

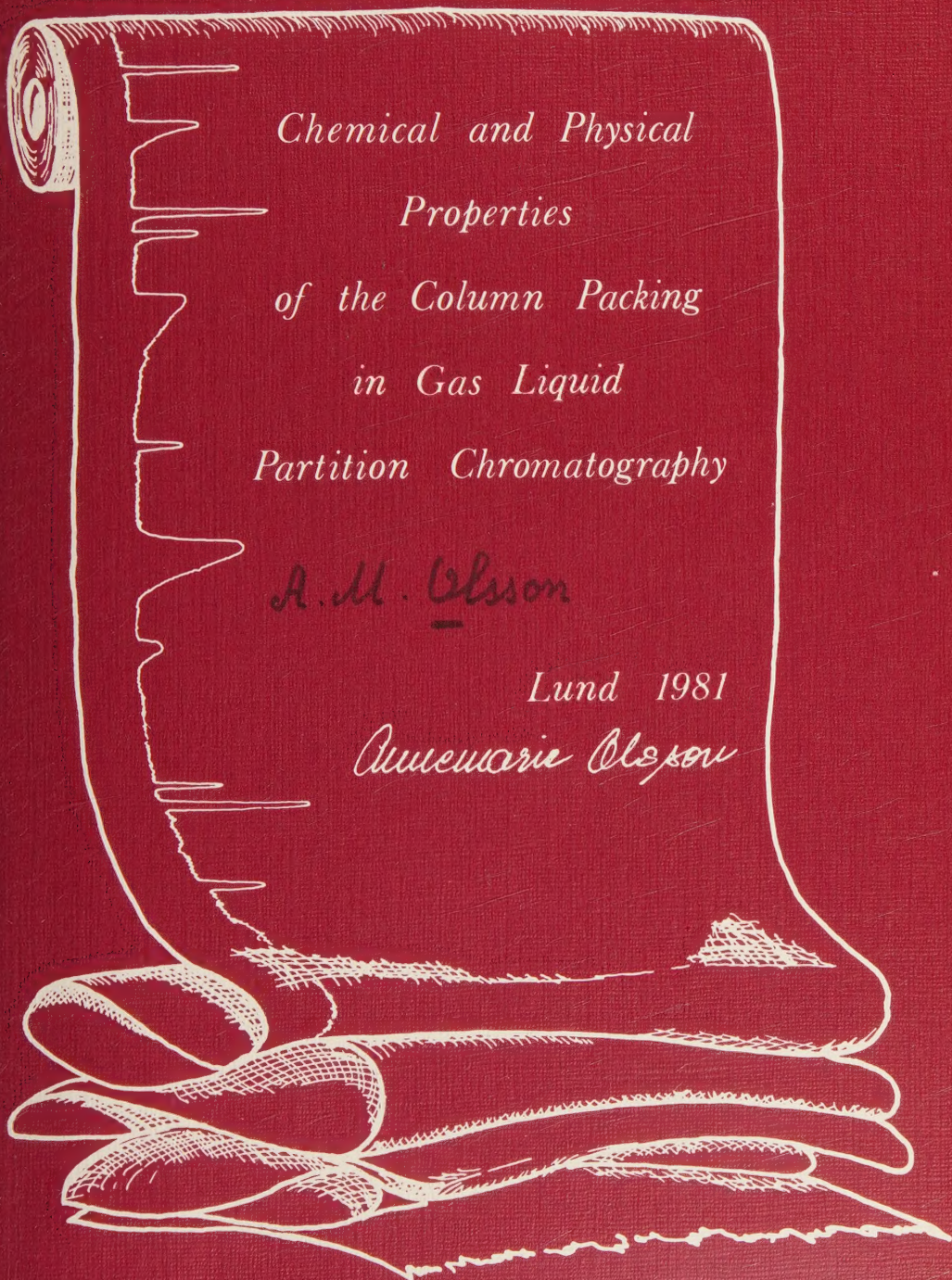
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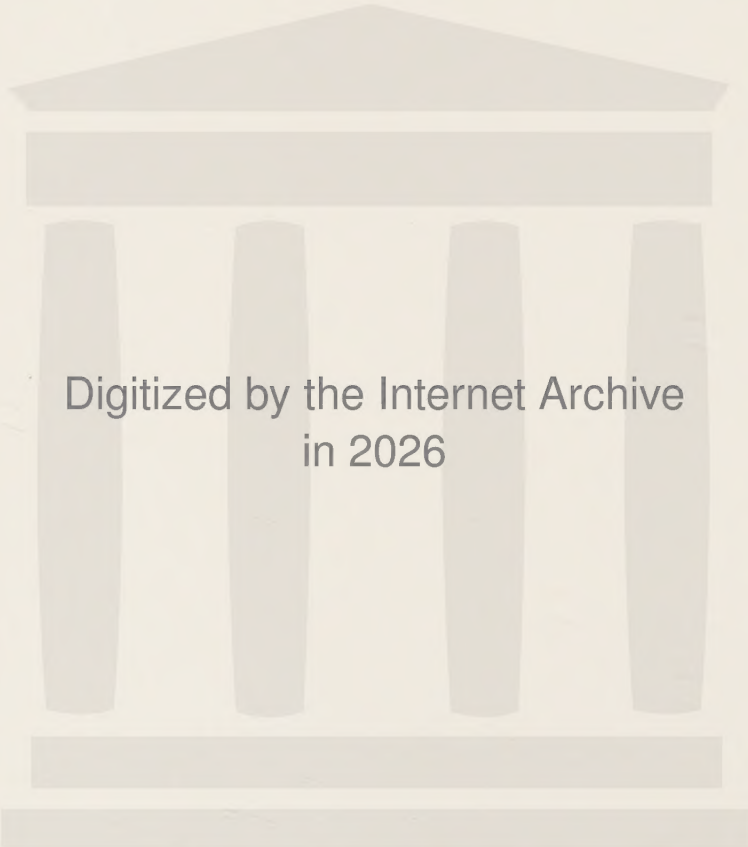
*Chemical and Physical
Properties
of the Column Packing
in Gas Liquid
Partition Chromatography*

A. M. Olsson

Lund 1981

Annemarie Olsson





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CHEMICAL AND PHYSICAL PROPERTIES OF THE COLUMN PACKING

IN GAS LIQUID PARTITION CHROMATOGRAPHY

by

Anne-Marie Olsson, fil.mag., Hb



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Abstract Mixed retention mechanisms in gas chromatography were studied for solute-solvent systems with pronounced adsorption effects; polar solutes on n-octadecane columns, n-alkanes on 3,3'-oxydipropionitrile (ODPN) columns and amines on columns with Carbowax 20 M + alkali. As a consequence of mixed retention mechanisms the Kovats' retention indices varied considerably and correlation between retention indices and structural differences can thus be misleading. An unexpected reaction between Carbowax 20 M and potassium hydroxide is commented. A static technique for the determination of the partition coefficient was developed. By utilizing pure partition equilibria, the linear decrease of solute retention volume, with accumulated carrier gas volume through the column, was used to correct for column bleeding. From the same relationships, a method was developed to determine very low vapour pressures, applied to n-octadecane, ODPN, 1-chloroalkanes and some pheromones. Different methods for the determination of the amount of the liquid loading were applied with an extended investigation of the evaporation method with the use of a thermogravimetric balance. Through investigation of the amount of the liquid phase along the column an unequal distribution was obtained. The inlet end of the column is depleted of the stationary phase, while in other parts of the column the liquid loading is increased due to gravitational settling.			
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Date 8 dec 1981

CHEMICAL AND PHYSICAL PROPERTIES OF THE COLUMN PACKING IN
GAS LIQUID PARTITION CHROMATOGRAPHY

This thesis summarizes the work presented in the following papers, referred to in the discussion by the Roman numerals I-VII

- I Sensitivity of Retention Index to Variations in Column
Liquid Loading and Sample Size.

L. Mathiasson, J.Å. Jönsson, A.M. Olsson and L. Haraldson,
J. Chromatogr. 152(1978)11.

- II Mixed Retention Mechanisms in Gas-Liquid Chromatography.
V. Studies of Alkali Treated Carbowax Columns for Amine
Analysis.

A.M. Olsson and J.Å. Jönsson in Advances in Chromatography
1981, (Ed. A. Zlatkins), Chromatography Symposium, Houston,
TX, 1981, p. 149.

- III A Static Technique for the Determination of Partition
Coefficients by Gas Chromatography.

A.M. Olsson, Acta Chem. Scand. A34(1980)699.

- IV Determination of the Loss of Stationary Phase in Gas-Liquid
Chromatography from the Change in Retention Volume.

A.M. Olsson, L. Mathiasson, J.Å. Jönsson and L. Haraldson,
J. Chromatogr. 128(1976)35.

- V A GLC-method for the Determination of Low Vapour Pressures
Applied to 1-Chloroalkanes.

J.Å. Jönsson, L. Mathiasson and A.M. Olsson,
Acta Scand. Chem. A34(1980)147.

VI Determination of the Vapour Pressures of some Moth Sex Pheromone Components and Related Compounds by a Gas Chromatographic Method.

A.M. Olsson, J.Å. Jönsson, B. Thelin and T. Liljefors,
in manuscript.

VII Distribution of the Stationary Phase in the GLC-Column.

A.M. Olsson and J.Å. Jönsson, Submitted to J. High Resol.
Chromatogr. Chromatogr. Commun.

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1. INTRODUCTION

Among all the different types of chromatography, the most widely used in the two last decades is gas liquid chromatography, GLC, invented in 1952 by James and Martin [1].

The solute is partitioned between a liquid as the stationary phase and a gas as the mobile phase. The partition coefficient, K_1 , of the solute, is defined as the concentration ratio

$$K_1 = \frac{c_l}{c_g} \quad (1)$$

where c_l is the concentration of solute in the stationary phase and c_g is its concentration in the gas phase.

The solute is retained in the column a time interval corresponding to the retention volume, V_R , that is the carrier gas volume needed to transport the solute through the column. According to chromatographic theory, the net retention volume, V_N , is related to the partition coefficient as

$$V_N = V_R - V_M = K_1 \cdot V_l \quad (2)$$

where V_M is the volume of the empty space in the column (the void volume) and V_l is the volume of stationary liquid phase in the column. The volumes V_N , V_R and V_M are all measured at the column temperature and corrected for the pressure drop in the column.

The derivation of this equation rests on several assumptions [2]. One of the most important is that the retention of a solute is caused only by dissolution in the liquid phase behaving as a bulk liquid, whose properties are not affected by its distribution on

the support. In this ideal case the concept gas liquid partition chromatography, GLCP, is used. The effects of other possible retention equilibria, such as adsorption of solute at the gas-liquid and at the gas-solid interfaces, is interpreted in chapter 3.

Retention volumes are influenced by the column temperature, since the partition coefficients are temperature dependent. The variation in the value of the partition coefficient with the column temperature is related to the enthalpy of solvation, ΔH_s , of the solute as

$$\frac{d \ln K_1}{dT} = \frac{\Delta H_s}{R \cdot T^2} \quad (3)$$

under the assumption that K_1 is an approximation of the thermodynamic partition coefficient, i.e. the ratio of activities.

From eq. (2) and (3) it can be seen that the retention volume of a compound is easy to vary by changing the temperature or the volume of the stationary phase or by using a different liquid phase.

The theory of GLC is relatively uncomplicated and the retention of a compound can easily be varied. Furthermore, the wide variety of liquids that can be used, as the stationary phase, to obtain the desired selectivity have contributed to the wide spread use of GLC. It is also the chromatographic technique that has been best studied theoretically.

Mainly the basic knowledge about the column packing is considered in this study. The choice of stationary phases was governed by the purpose to exaggerate different effects. The stationary phases with widely varying polarities in chapter 3 were selected for the study of the negative influences of mixed retention mechanisms when pure GLC is attempted. It is true that in practical applications the simultaneous occurrence of bulk solution and adsorption can

often be utilized to enhance the column efficiency and selectivity [3]. This approach, gas liquid solid chromatography was not investigated in this work, even if the adsorption tendency of amines described in section 3.3 is a closely related subject.

Throughout this work a diatomaceous silica support was used. The white type, Chromosorb W, was chosen due to its lower adsorptivity compared with the pink type. In spite of this, undesirable adsorption effects are obtained for polar, especially hydrogen-bonding, solutes. This is due to the presence of silanol and siloxane groups and impurities, mainly metallic oxides, in the surface, acting as proton donors or acceptors. In a review by Ottenstein [4] a more comprehensive information on the structure and the properties of solid supports is available.

2. MIXED RETENTION MECHANISMS

2.1 Theoretical Background

The retention of a solute in a GLC column is principally always the result of mixed mechanisms. The partition between the gas phase and the bulk liquid phase is normally the primary retention contribution. The other contributions originate from adsorption equilibria, adsorption at the gas-liquid interface and on the support surface itself.

With all the three mechanisms present, the retention volume can be represented by the wellknown equation

$$V_N = K_1 \cdot V_1 + K_I \cdot A_I + K_S \cdot A_S \quad (4)$$

verified and developed in various forms by many scientists [5]

from the first suggestion of Martin 1961 [6]. The first term of the right-hand side of the equation express the partition contribution as in equation 1. The two following terms account for the adsorption contributions, where A_I and A_S are the area of the liquid phase surface and the area of the support surface, respectively, and K_I and K_S are the adsorption coefficients. This equation is strictly valid only at infinite dilution and to reach the linear part of the interfacial adsorption isotherms, where K_I and K_S are constant, a great demand upon small concentrations of the analyte is required. Especially for polar solutes on a non-polar stationary phase, the contribution from adsorption varies very strongly with the amount of analyte, as shown in figure 1.

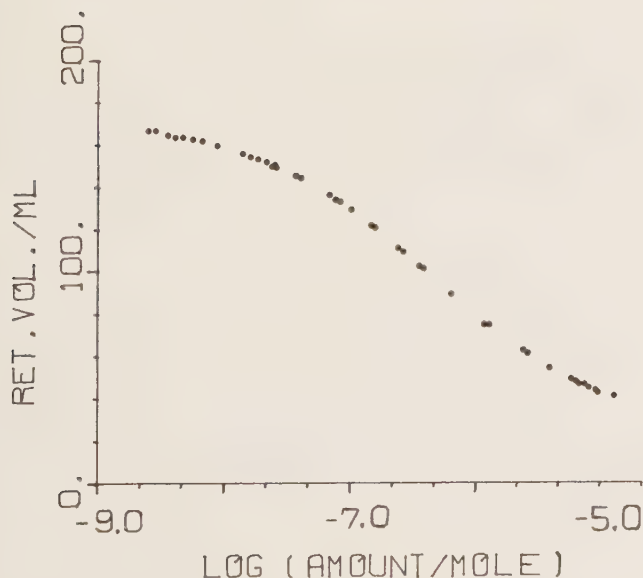


Fig. 1. Retention volume vs amount of sample for ethyl methyl ketone on a 10% n-octadecane column.

The usefulness of equation 4 is thus of limited use for the interpretation of experimental data. For instance, to evaluate the partition coefficient, K_L , - a requirement for most physico-chemical measurements -, an extrapolation to infinite dilution is necessary. According to Conder and Young [7] very few systems have been shown to be free of mixed retention mechanisms. One example is hydrocarbon solutes in hydrocarbon solvents, which is applied in paper IV.

Accurate measurements of retention data, with respect to the partition, requires therefore the retention volumes to be corrected for the adsorption. A very helpful tool is the equation for non-linear adsorption (finite amount of analyte)

$$V_N = A + \frac{C}{(1 + B \sqrt{\frac{n_t}{V_N + V_M}})^2} \quad (5)$$

derived by Jönsson and Mathiasson [8]. V_N is the net retention volume and V_M is the void volume. The first term A of the right-hand side of the equation represents the contribution from bulk partition, $K_L \cdot V_L$, independent of the amount of analyte. The second term expresses the contribution from adsorption with n_t as the injected amount of analyte. At infinite dilution, i.e. when n_t tends to zero, the adsorption contribution is constant and equal to C. The theoretical considerations and the fact that only one adsorption isotherm is present, compared with two as in equation 4, are described in ref. 8, 9.

A typical use of the equation is shown in figure 2. The analyte is ethyl methyl ketone covering a wide range of injected amounts, and the equation is fitted to the experimental data (solid line).

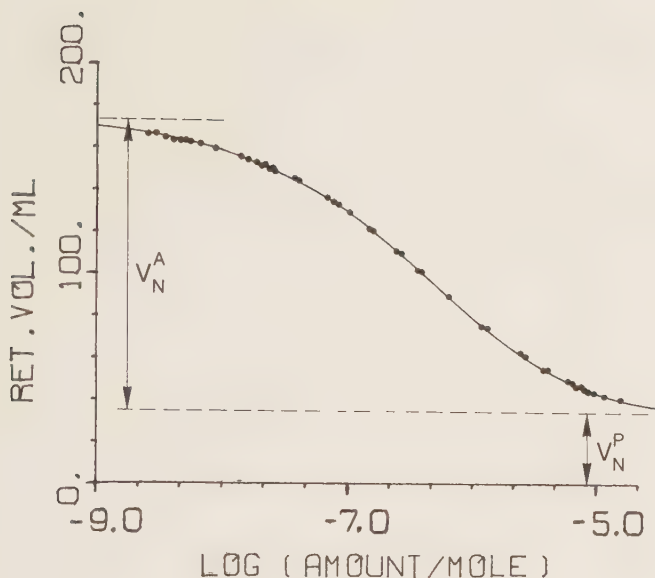


Fig. 2. Fitting of eq. 5 to the experimental points of ethyl methyl ketone on 10% n-octadecane column. V_N^A and V_N^P are the contributions from adsorption and partition to the retention volume, respectively.

At a given amount of analyte, the contributions from adsorption, V_N^A , and partition, V_N^P , are obtained directly. In this case, the constants A and C give an adsorption contribution of 80% of the total retention volume at infinite dilution. In papers I and II, most of the discussions are based on the results from the fitting of this equation.

2.2 Polar solutes in a non-polar stationary phase

The term 'polar' has been used in the same sense as in ref. 10 throughout this work. It characterizes the affinity of a component for its environment, but it is primarily based upon the dipole

moment of the molecule. The polarity of a compound is a relative concept and can vary depending on the mixture in which it is studied.

The polar solutes, ethyl methyl ketone and diisopropyl ether, were injected in a wide range of sample sizes on n-octadecane columns with loadings between 5 and 25%. As can be seen in Fig. 1, paper I, the retention volume of diisopropyl ether varies considerably with the amount injected, and for ethyl methyl ketone this variation is even larger. This behaviour confirms that adsorption contributes to the retention, but this contribution is smaller as the amount of the analyte is increased. Furthermore, with n-alkanes as solutes, the plot of the net retention volumes versus the amount of analyte is a straight line as expected, i.e. without adsorption effects.

The adsorption contribution is not only influenced by the sample size, but as well by the liquid loading. The higher liquid loading, $> 5\%$, the less adsorption effects. This is in agreement with the results of Mathiasson and Jönsson [9], who also demonstrate that for loadings of less than 5% the situation is more complicated. However, most of the adsorption contribution could be attributed to the gas-liquid interface for loadings higher than 6%, but to the gas-solid interface for loadings less than 0.5%. These statements are based upon a postulated drop-model [9] for the distribution of the stationary phase. Thus, the strong adsorption effects of ethyl methyl ketone and diisopropyl ether are considered to be mainly due to adsorption at the surface of n-octadecane. The influence on retention indices from the adsorption effects are discussed separately below.

In addition, according to ref. 11, 12, the stationary phase, n-octadecane, does not wet the silanized support Supasorb, but is distributed as droplets, leaving much of the surface uncovered. As discussed on page 3, this surface still retains many adsorption sites for polar solutes. Consequently, some of the adsorption effects are presumably due to this fact.

2.3 Polar, hydrogen-bonding solutes in a polar stationary phase

The solutes investigated were different classes of amines. They represent a type of compounds difficult to separate and even worse to quantify at trace levels. As they have an alkaline character, all acidic adsorption sites in the column are mischievous. To neutralize those, an addition of alkali to a polar stationary phase is frequently used.

In paper II the adsorption effects are studied on Carbowax 20 M columns with an amount of KOH between 0 and 5%. As expected, the tailing of the amine peaks decreases drastically upon the first small addition of KOH, but levels off already at 2% of KOH. This is illustrated in Fig. 1, paper II, for the solute piperidine, where a larger retention volume implies a larger adsorption contribution and consequently a more tailing peak. In those discussions it should be emphasized how important the amount of the analyte is. This great influence explains certainly many conflicting results from studies of mixed retention mechanisms, in general.

Another feature of paper II is the large difference in adsorption effects using silanized or non-silanized supports. From Fig. 5 it may be concluded that unsilanized support is far more favourable than silanized, and this choice is even more important than the

addition of KOH in reducing adsorption effects. This is in contradiction to the general rule which states that, when polar and especially, H-bonding solutes are involved, a silanized support is desirable to reduce surface activity. However, as mentioned before, a silanized support is poorly wetted, with the stationary phase distributed as droplets, resulting in a larger area of the gas-liquid interface. This might be one source of larger adsorption effects at supports treated with dimethyldichlorosilane (DMCS). The main reason, however, is most probably the better deactivation of the support by Carbowax 20 M [13,14].

The same columns were also investigated with respect to water as solvent. The water is strongly adsorbed and the higher content of KOH the more tailing and broadening of the water peak is obtained.

2.4 Non-polar solutes in a polar stationary phase

Regarding the analyte, we now have a reversed polarity situation compared to the two previous cases. This was the first type of systems found [6] to possess mixed retention mechanisms and it is also the most well established, with few contradictory results.

Ten solutes, covering the whole polarity scale were investigated on 3,3'-oxydipropionitrile (ODPN) as the stationary phase in paper I. The alkanes showed observable adsorption contributions, but independent of the amount of analyte. When the retention volumes are plotted versus the liquid volume, regression lines with significant positive intercepts are obtained (Fig. 6, paper I). Thus for each loading of ODPN a constant contribution is observed and is interpreted as due to adsorption at the surface of the liquid phase as suggested by Martin [6] and others [15]. Furthermore, with a

loading of 10% ODPN the liquid phase is most probably distributed as a layer. For n-octadecane [9] the liquid phase is distributed as droplets below 6% loading. Increasing the loading from 6% to 10% results in the formation of a homogeneous layer and a further increase from 10% to 25% results in smoothing of cavities and filling of larger and larger pores. Thus, the surface area of the liquid phase will slowly decrease with increasing loading, resulting in a lower adsorption contribution relative to partition at higher loadings.

For the other solutes, only ethanol showed significant adsorption effects but, in contrast to the alkanes, the retention volume varies with the amount of ethanol. This is probably explained by its H-bonding capacity, coherent with results for ethanol on Carbowax 20 M + KOH columns.

2.5 Retention related to molecular structure

One of the most important tasks in gas chromatography is to correlate retention data with the molecular structure of the solute - stationary phase system, with the purpose of either evaluating thermodynamic properties or simply facilitating the choice of the stationary phase.

Different approaches [16], all related to the concept polarity, have been proposed. Kovats' retention index system [17], comparing the retention of the solute with the retention of an homologous series of n-alkanes, is still the most widely used criterion. However, as the n-alkanes are used as the "fixed" points, unequivocal values can only be obtained with non-polar solutes on

non-polar stationary phases. For other systems, the indices can vary considerably with the amount of analyte, with the liquid phase loading or/and with the type of support, due to mixed retention mechanisms.

These influences are studied in paper I. For the polar solutes, ethyl methyl ketone and diisopropyl ether on n-octadecane columns, the indices varied as much as 350 and 130 index units (Fig. 4, paper I), respectively. In the opposite case, i.e. on polar ODPN columns, it is the use of the non-polar n-alkanes as the references which cause the variation of 40-100 index units (Fig. 7, paper I).

As a consequence, correlation between retention indices and structural differences can be misleading, when mixed retention mechanisms are present. Thus, there is a need of another approach, less fraught with ambiguities, related to physical properties. This has also been attempted by Novák and coworkers [18] in 1973, by relating polarity to the partial molar excess Gibbs free energy of the solvent per unit of CH_2 as the solute. Two successive acetate homologs were recommended as solutes or testing substances. Although new approaches have been developed [19,20] neither of them have achieved such wide acceptance as the Kovats' Index.

2.6 Alkali-treated Carbowax 20 M columns

In paper II, the use of alkali in reducing the substantial adsorption tendency of amines have been studied. It was observed that an excess of alkali has to be added, far more than the amount needed, to neutralize the acidic adsorption sites of the support. No plausible explanation could be found in the literature. It was considered hardly possible that Carbowax 20 M and KOH would react [21]

but detailed studies have shown the occurrence of the reaction.

A slow reaction takes place when Carbowax 20 M and KOH are mixed. At 80°C, in a couple of days, the colourless mixture gradually becomes a yellow-brown polymer. This reaction takes place whether in inert dry environment (under nitrogen) or in the ambient.

By now, it was not possible to establish the mechanism of the reaction. Acid titration reveals that the excess of KOH decreases exponentially with the time. I.R. spectroscopy was unable to show differences between Carbowax 20 M and the end product. In the column this reaction takes necessarily place but it is difficult to know to what extent. Catalytical activity by the support, as pointed out by Berezhkin [22], can influence. This actual reaction could be the reason of the necessary excess of alkali, and the influence from this transformation of the stationary phase on the retention of the amines is not yet known.

3. PURE PARTITION EQUILIBRIA

3.1 Determination of the Partition Coefficient

So far all the conclusions have been obtained from the fitting of the eq. 5. The contribution of the adsorption is then determined, once the contribution from the partition has been ascertained. The K_L -value thus obtained is a constant, which verifies the assumption that the dissolution of the analyte in the liquid phase is ideal, with a constant contribution to V_N of $V_1 \cdot K_1$ over the different amounts of analyte studied. This was desirable to verify by means of some independent method.

For three solute-solvent systems (ethyl methyl ketone, diisopropyl ether and n-pentane with n-octadecane as the common solvent) a static technique was developed to determine the partition coefficient. This technique is described in paper III, where the determinations of the partition coefficients are directly based upon the definition of K_1 (eq. 1, page 1). Both concentrations of the gas- and liquid- phases are determined simultaneously, in relation to each other. No correction for the possible loss of analyte adsorbed within the equilibrium system is thus required. Moreover, the area of the liquid phase surface is strongly reduced, at least 250 times, in comparison to the area in the column.

The results obtained from both techniques showed excellent agreement (Table 2, paper III). The main advantage of the static technique is the simplicity of the equipment. As seen from the results in Table 1, paper III, a good precision was obtained (standard deviation of the mean from 0.3% to 1.2% with 95% confidence). Transfer of the phases from the equilibrium vessel to the gas chromatograph requires, however, some technical skill.

3.2 Bleeding of the stationary phase

Hitherto the bleeding of the stationary phase has not been taken into consideration. For most analytical applications of GLC bleeding can be ignored. For those cases, however, when significant effects due to bleeding are expected, a short pre-column, with the same stationary phase as the main column, can be used. This technique was introduced by Kwantes and Rijnders [23] for n-octane as stationary phase. Although, with this approach, the bleeding rate will be kept very low, the loss cannot be completely eliminated. Furthermore, a new uncertainty is introduced in the calculation of the mass of the stationary phase. This

approach is inadequate when employing a high-precision gas chromatograph (see Equipment, page 23) with a great demand upon long-term stability for the whole system.

Without the precolumn, the mass of the stationary phase will decrease and cause a significant downward drift in retention volume during operation. Keller et.al. [24,25] have suggested that the retention volume of a solute could be used to correct for weight losses from the stationary phase in GLC. The same approach was also later utilized by Langer and coworkers [26] with a graphical technique. In paper IV a further development is described and equations for correction of the loss of the stationary phase are derived. Bleeding measurements have also been utilized in a method for the determination of very low vapour pressures of compounds used as stationary phases. The method has been applied in papers V and VI. This will be discussed in the next section.

For stationary phases, consisting of only one substance, the retention volume decreases linearly with the total volume of carrier gas, V_{acc} , which has passed through the column (Fig. 2, paper IV). The slope, dV_N/dV_{acc} , is a measure of the bleeding rate. With a new column, i.e. when no carrier gas has yet passed through ($V_{acc} = 0$), the retention volume, V_N^B , is given as the intercept at the ordinate. The starting volume of the liquid phase, V_L^B , is the volume of the liquid phase used in preparation of the column.

The linear decrease is only observed if the assumption that partition is the only mechanism responsible for solute retention, is valid. Then the volume of the stationary phase can be calculated from

$$V_1 = V_1^B + \frac{dV_N}{dV_{acc}} \cdot \frac{1}{K_1} \cdot V_{acc} \quad (6)$$

where K_1 is determined as V_N^B/V_1^B .

A simplified method can be used, if the value of the saturation vapour pressure, p , of the stationary phase at the actual temperature, T , can be found in the literature. Then the following relationship applies

$$\frac{dV_N}{dV_{acc}} \cdot \frac{1}{K_1} = -p \cdot \frac{M}{R \cdot T \cdot \delta} \quad (7)$$

where M is the molecular weight and δ is the density of the stationary phase. Hereafter, the volume of the liquid phase is again calculated from eq. 6. Unfortunately very few values of p are available for the compounds used as stationary phases.

The former procedure was applied to the stationary phases *n*-octadecane and 3,3'-oxydipropionitrile (ODPN). As both of these stationary phases are fairly volatile and the experiments in paper I are time consuming, this correction was necessary. *N*-heptane was used as solute in *N*-octadecane and butyl acetate in ODPN. These solutes have negligible adsorption effects in their stationary phases, respectively.

In the case of Carbowax 20 M (paper II) the changes appear to be more complex than can be accounted for by simple physical evaporation, according to Keller and coworkers [24]. Furthermore, with the addition of alkali the packing became undefinable (page 12)

Therefore no correction was made for the column bleeding in paper II. Anyhow, the results would not be influenced, because the adsorption effects of the amines are overwhelming.

3.3 Determination of Low Vapour Pressures

The vapour pressure, p , of the stationary phase can be determined at a given temperature, T , from

$$p = -\frac{dV_N}{dV_{acc}} \cdot \frac{W_1^B}{V_N^B} \cdot \frac{R \cdot T}{M} \quad (8)$$

where W_1^B (equal to $V_1^B \cdot \delta$) is the mass of the stationary phase when the column is packed. The other symbols are the same as above.

As eq. 6 and eq. 7 this expression is valid when the effects of the adsorption on the retention volume can be neglected. This can easily be achieved by proper selection of solute. The use of inert supports with high stationary phase loading (e.g. 20% w/w) also minimizes adsorption effects.

The high liquid loading also minimizes the Kelvin Effect [27]. This is due to variations in surface tension and results in: (a) the vapour pressure of a liquid is greater when it is in the form of small droplets than when it has a plane surface, or (b) the vapour pressure is lowered over a concave surface. As mentioned above, page 10, the droplike arrangement of the liquid phase should be transformed into a layer long before 10% of liquid loading. Consequently (a) can be neglected. Smoothing of cavities and filling of larger pores are enhanced after this stage, but proceed already from the first addition of liquid phase. This process minimizes effect (b) and the reduction in vapour pressure can be estimated [28] in the range of 0.2% for the liquids studied.

The method was applied to n-octadecane and 3,3'-oxydipropionitrile in paper IV, to 1-chloroalkanes in paper V and to some pheromones in paper VI. Pheromones are defined as substances which are secreted to the outside by an individual and received by an individual of the same species, in which they release a specific reaction, for example, a definite behaviour or a developmental process [29]. In this case the pheromones are sex attractants for moths. As seen from the applications, the method is preferably adapted for the determinations of low vapour pressures (1-100 Pa). The limitations at higher or lower vapour pressure are discussed in paper V.

The results are independent of carrier gas flow rate between 25 and 85 ml/min and indicates that the carrier gas is saturated with the vapour of the stationary phase (paper IV). This is also confirmed by the good agreement between the values obtained in paper IV and V and the available literature values. Consequently, it is the true vapour pressure at the actual column temperature that is determined.

The imprecision of the method is mainly governed by the uncertainty in the slope dV_N/dV_{acc} of the regression line. This uncertainty is easily diminished by increasing the number of points for each regression line. In paper VI each experiment was continued until the uncertainty (95% confidence intervals) was less than 1% approaching the uncertainty of w_1^B . The imprecision of the vapour pressures in paper VI are therefore calculated with respect to the uncertainties of all the factors in eq. 8. For the vapour pressures in paper IV and V the imprecisions are totally dominated by the uncertainties of dV_N/dV_{acc} .

The reproducibility was checked by repeated determinations of several vapour pressures in paper VI. It was confirmed that the new values lie well within the confidence intervals of the former. For each determination new columns were used. Impurities in the original liquid have been excluded as a source of error. They obviously influence the vapour pressure, but differently depending on their relative vapour pressures. Those with higher vapour pressure are easily detected and also easily corrected for. More detailed discussions are given in paper V.

4. CONTENT AND DISTRIBUTION OF THE STATIONARY PHASE IN THE COLUMN

In the study of physical and chemical properties with gas chromatography, the knowledge of the amount of the stationary phase is essential. Several methods for the quantitation of the stationary phase have been reported. The importance of accurate values for the liquid phase volume, V_1 , have been thoroughly emphasized and as late as 1978 Laub and coworkers [30], in a study of error analysis of thermodynamic measurements, concluded that V_1 is still the largest source of random error. Other sources of error arising from, for instance, the determination of retention times or volumes, were found to be comparatively negligible.

In the course of this work, three methods have been employed: weighing at the preparation of the column, combustion and/or evaporation with conventional oven or evaporation with a thermogravimetric balance and, finally, extraction and gravimetric determination of the stationary phase. If the bleeding is measured as described in 3.2 the data thus obtained give an excellent check of the stationary phase determinations.

For all columns the amount of stationary phase used in the preparation of the column has been determined by weighing. An excellent description of the procedure is found in ref. [31].

With the two following procedures, the amount of the stationary phase at the end of the column lifetime is determined. Petsev and coworkers [32] briefly compared the various methods and concluded that evaporation is very reliable even when employing a simple experimental technique - heating of the packing in a crucible in the air. In this work, this technique was employed with a thermogravimetric balance (Mettler TA-HE 20). With this balance an important advantage is offered since the weight loss is continuously followed. The end temperature and the rate of increase of the temperature are then adequately chosen from weight loss curves.

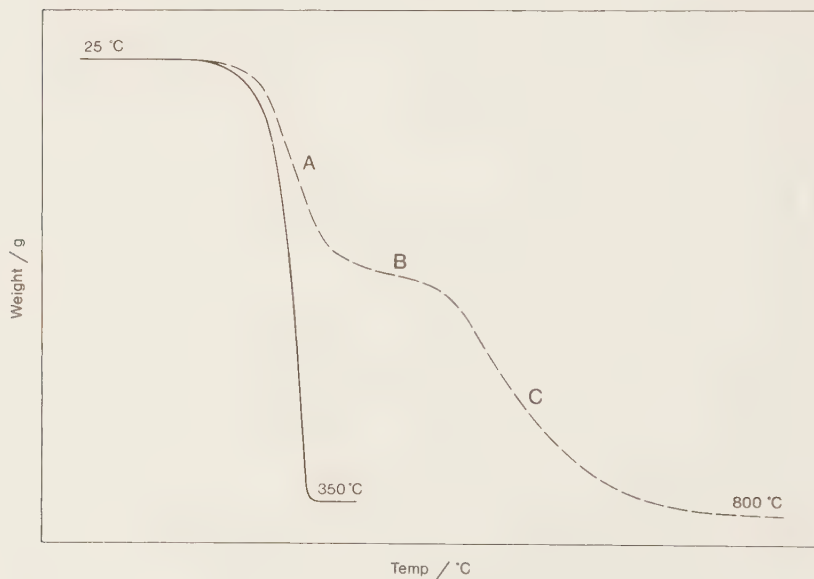


Fig. 3. Thermogravimetric measurements of weight loss patterns for chromatographic packings, n-octadecane on silanized support, with the temperature.

Different loadings of n-octadecane on silanized supports were studied [33]. The samples were heated in an inert stream of nitrogen. A high end-temperature causes the silane groups on the support to be evaporated together with the liquid phase. This is illustrated by the dashed curve in Fig. 3, where the first weight loss (A) corresponds to evaporation and the plateau (B) to degradation of the liquid phase into less volatile compounds which eventually disappear (C) together with the silane groups. The loss of the silane groups seems to begin at about 550-600°C. With this procedure a separate determination of the weight loss, due to the silane groups on pure support material, is therefore required.

The end-temperature can also be set well below 550°C, still enough for a complete evaporation of the stationary phase, but unaffecting the silane groups. For n-octadecane, 350°C was found to be sufficient but it is important to keep a low rate of increase of temperature (5-10°C/min) to avoid the degradation. The weight loss curves show a decrease to a well defined constant value before the end-temperature is reached. A typical pattern is shown as the solid curve in Fig. 3.

For both cases, the water content in the packing has to be separately determined. It was, unfortunately, not possible to distinguish between the evaporation of water and of the n-octadecane.

The latter described procedure was found to give better precision and for three samples from the same packing the results were 19.67%, 19.70% and 19.66%. This variation is of the same order as the stability of the balance. The accuracy was checked by comparing the results with data from weighings at the preparation, Table 1, and as can be seen a good agreement is obtained.

Table I. Amount of loading of n-octadecane on silanized support determined thermogravimetrically and by weighings at the preparation of the packing.

Amount of loading (%)	
Thermogravimetric	By weighings
19.97	19.91
15.01	14.92
10.09	10.04
7.34	7.40
5.01	5.02

The thermogravimetric method is of special value for the choice of appropriate operating conditions when simpler equipment is employed.

Finally, in paper VII another method, extraction, was applied to the pheromone, (E)-5-decenyl acetate, column. The amount of pheromone was calculated from careful weighings of the packing before and after extraction with n-hexane in a Soxhlet extractor. This procedure was regarded to have sufficient accuracy and precision for a loading of 10% of the stationary phase. For low-loaded columns a gas chromatographic determination of the amount of stationary phase in the extract is preferable [34]. Another feature of using GC as detection system is the possibility of determining the composition of mixed stationary phases as long as they are volatile enough for GC. Another approach is to weigh the amount of the stationary phase extracted from the packing [35].

If determinations are made both at the beginning, V_1^B , and at the end, V_1^E , of the column lifetime, the loading at a particular time can be estimated only if the column is used at a single temperature.

When the stationary phase loading is determined either at the beginning or at the end of a column lifetime, the amount of stationary phase at any other time can be determined from bleeding data irrespective of any changes in column temperature or carrier gas flow rate.

A great advantage by utilizing bleeding data is the excellent possibility of checking the accuracy and precision of the value of V_1^B obtained at the preparation of the column. A calculated value of V_1^B is generated from the bleeding data combined with V_1^E , where V_1^E is determined by one of the methods described above. Furthermore, the agreement in vapour pressure values between different preparations of the same column (Paper VI) is also a test for the determination of V_1^B , provided that the bleeding of the stationary phase is prolonged until the imprecision of the vapour pressure value is dominated by the uncertainty of the amount of liquid (page 17). Summarizing, the imprecision of V_1^B from weighing data at the preparation of the column is estimated to be less than $\pm 0.5\%$ and the inaccuracy in the order of 0.6% for loadings between 5% and 20% .

The distribution of the stationary phase in the GLC-column is investigated in paper VII. The phase distribution along the column and not at each support grain is under consideration. Column efficiency is negatively affected by inhomogeneities of the stationary phase coating along the column. Furthermore, uneven film thickness may invalidate some assumptions related to the theory of HETP. Prolongated use of a column led to a depletion of the stationary phase in the inlet end of the column, in agreement with the conclusions of Keller and coworkers [24,25]. The depletion was also suggested by Pscheidl [36] who was not able

to obtain reproducible data in kinetic studies. The reason is the bleeding of the liquid phase. Rapid evaporation of the phase takes place in the beginning of the column where the carrier gas is saturated. As a consequence, uncovered support patches can be created, increasing adsorption of the analyte and impairing the resolution. It should be mentioned that if the temperature of the injector end is above the operating temperature of the column (as with ordinary gas chromatographs but not in the present work) the described effect is aggravated. The same effect will also be observed at the detector end of the column.

Another process, which results in an uneven distribution by increasing the film thickness, is by the redistribution of the stationary phase due to gravitational settling. The V-formed columns used in this study allow this effect to be easily accessible but the same conclusions are valid for spiral columns.

5. EQUIPMENT

A high precision gas chromatograph was used for the majority of the measurements. This gas chromatograph is coupled on line with a mini computer and is mainly developed by Jönsson [37-39]. It enables direct measurement of retention volume without involving the concept of retention time. Several sensors measure the relevant pressures and flow rate at short time intervals (every 100 ms). From these values, a volume scale is calculated and the value of the carrier gas volume, that has passed through the column, is thus continuously known. With this procedure a higher precision is obtained, than with the classical method, since the measurements rely only on the stability of the devices which are used to measure the pressures and the flow rate, and not on the stability of the flow regulation.

The measurements are always running when gas is flowing through the instrument. Thus it is possible to keep a record of the total amount of gas which has passed through a column since its installation. This enables the column bleeding to be accounted for (section 3.2), as the equipment also has a very good long-term stability. Furthermore, the inaccuracy of the measurement of the carrier gas volume is estimated to be as low as 0.15% and the imprecision even less.

For symmetrical peaks the imprecision of the retention volume can be as small as 0.02%. This is approximately the variation of the partition coefficient due to fluctuations of the column temperature, kept below 0.01°C by means of an oil bath.

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LIST OF SYMBOLS

A	Contribution to retention from liquid solution, equal to $K_1 \cdot V_1$
A_I	Surface area of the stationary liquid
A_S	Surface area of the solid support
B	Empirical constant
C	Adsorption contribution to retention at infinite dilution
c_g	Solute concentration in gas phase
c_l	Solute concentration in liquid phase
ΔH_S	Enthalpy of solvation, equal to the sum of enthalpy of solution and condensation
K_I	Adsorption coefficient for solute between liquid surface and gas phase
K_l	Partition coefficient for solute between gas phase and bulk liquid phase
K_S	Adsorption coefficient for solute between solid support surface and gas phase
M	Molecular weight
n_t	Total number of injected molecules
p	Saturated vapour pressure
R	Gas constant
T	Temperature
V_l	Volume of the liquid stationary phase in a column
V_l^B	Volume of the liquid stationary phase in a column at the beginning of an experiment
V_l^E	Volume of the liquid stationary phase in a column at the end of an experiment
V_M	Volume of the gas phase in the column (void volume) at column temperature, corrected for the pressure drop
V_N	Net retention volume at column temperature, corrected for void volume and the pressure drop

V_N^A	Contribution to net retention volume from adsorption equilibria
V_N^B	Net retention volume at the beginning of an experiment
V_N^E	Net retention volume at the end of an experiment
V_N^P	Contribution to net retention volume from liquid solution
V_R	Retention volume at column temperature, corrected for the pressure drop
w_1^B	Mass of the stationary phase in a column at the beginning of an experiment
δ	Density of liquid stationary phase

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I



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SENSITIVITY OF RETENTION INDEX TO VARIATIONS IN COLUMN LIQUID LOADING AND SAMPLE SIZE

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SUMMARY

The reliability of retention index values in gas-liquid chromatography has been investigated. The liquid phase loading and sample size were varied on non-polar *n*-octadecane and highly polar 3,3'-oxydipropionitrile columns. It was found that these parameters greatly influenced the Kováts retention index for ethyl methyl ketone and for diisopropyl ether on *n*-octadecane columns, the variation being as great as 300 units for the former and 140 units for the latter. For the 3,3'-oxydipropionitrile columns the variation of retention index with sample size was small for all solutes except ethanol, where it was significant. Variation of the column loading had large effects on the Kováts retention indices for all solutes. It was concluded that both column loading and sample size ought to be high in order to keep the variation in retention indices as small as possible.

INTRODUCTION

Many articles dealing with the retention of a substance relative to one or more reference solutes have appeared since the 1950s. Recent surveys have been published by Ettre¹⁻³ and Haken⁴. Published retention data obtained under apparently the same conditions often differ considerably, *e.g.*, results for the Kováts retention index for benzene on squalane columns at 373°K differ by about 60 units in different investigations^{5,6}. These differences have often been considered to depend on errors in the measurements of variables such as carrier gas flow-rate, retention time or gas hold-up time. Impurities in the stationary phase and the use of different batches of the stationary phase have also been mentioned as causes of divergent retention index values.

However, little attention has been paid to differences in retention indices as a result of mixed retention mechanisms. Retention indices are influenced by sample size, liquid phase loading and type of support if the retention of either the investigated solutes or the reference compounds is not due entirely to bulk solution. Under these conditions, all correlations between increments in retention indices and structural differences in investigated solutes are dubious.

We have investigated polar solutes on non-polar columns and non-polar solutes on polar columns, where, according to Martin⁷, Martire⁸, Pecsok and Gump⁹ and others, mixed retention mechanisms can be expected. Our aim was to estimate the importance of variations in sample size and liquid phase loading on the retention values. The Kováts retention index system¹⁰, based on the retention of homologous *n*-alkanes, was used for the calculation of retention indices.

EXPERIMENTAL

All measurements were made with a high-precision gas chromatograph coupled on-line with an Alpha LSI-2 mini-computer described elsewhere¹¹⁻¹³.

n-Octadecane and 3,3'-oxydipropionitrile (ODPN) were used as stationary phases on Supasorb (40-60 mesh), acid-washed and treated with hexamethyldisilazane (BDH, Poole, Great Britain), as the support. V-shaped glass columns (1000 × 4 mm I.D.) containing *ca.* 4 g of packing were used. The temperature was $333.15 \pm 0.03^\circ\text{K}$. Hydrogen was used as the carrier gas and methane was used for the determination of the void volume. The pressure drop across the column varied between 50 and 70 mmHg and the carrier gas flow-rate between 54 and 56 ml/min with the *n*-octadecane columns, and 60-85 mmHg and 56-58 ml/min with the ODPN columns. The number of injections for each solute was about 100-150 on the *n*-octadecane columns and 40-60 on the ODPN columns. The amount of packing material and the percentage of stationary phase were carefully measured during the preparation of the columns.

Vapour samples (containing methane) were automatically injected using the injection system described earlier¹⁴. The amount injected for each solute varied over 3-4 decades within the range $5 \cdot 10^{-5}$ - $5 \cdot 10^{-10}$ mole.

Ethyl methyl ketone, diisopropyl ether, *n*-pentane and *n*-heptane were used as solutes on the *n*-octadecane columns, except that with a 5% loading, where *n*-hexane and *n*-octane were used instead of *n*-pentane and *n*-heptane. *n*-Nonane, *n*-undecane, benzene, toluene, trifluorobenzene, acetonitrile, ethanol, ethyl methyl ketone, di-*n*-butyl ether and 1-chlorobutane were used on the ODPN columns. The detector response was calibrated by injection of 2 μl of dilute solutions in carbon disulphide with a Hamilton syringe. Corrections for bleeding of the stationary phase during the experiments were made as described earlier¹⁵. The column loadings used are given in Table I.

TABLE I
COLUMN LOADINGS

Column	Stationary phase	Approx. loading (%)	Liquid volume, V_L (ml)
1	<i>n</i> -Octadecane	5	0.2020
2		10	0.4022
3		15	0.6491
4		20	0.8560
5		25	1.1874
6	3,3'-Oxydipropionitrile	10	0.3100
7		15	0.5082
8		20	0.6828
9		25	0.9936

RESULTS

Kováts retention indices (I) were calculated with the equation

$$I = 100 \left(n \cdot \frac{\log V_{R_x} - \log V_{R_z}}{\log V_{R_{z+n}} - \log V_{R_z}} + z \right) \quad (1)$$

where V_{R_x} , V_{R_z} and $V_{R_{z+n}}$ are the retention volumes of the substance x of interest and n -alkanes with z and $z+n$ carbon atoms, respectively.

The retention volume corresponding to the peak maximum was used for the retention index calculations as it can be measured with better precision than the centre of gravity or the median of skewed peaks.

Octadecane columns

The alkanes showed no significant variation in retention volume with sample size. Therefore, an average value of the retention volume for each alkane could be used in further calculations. The retention volume of ethyl methyl ketone varied widely and that of diisopropyl ether moderately with sample size. The ethyl methyl ketone peaks showed very bad tailing, which became worse as the size of the injected samples became smaller. The diisopropyl ether peaks also showed some tailing. Fig. 1 shows the variation in retention volume for diisopropyl ether on the 15% n -octadecane column.

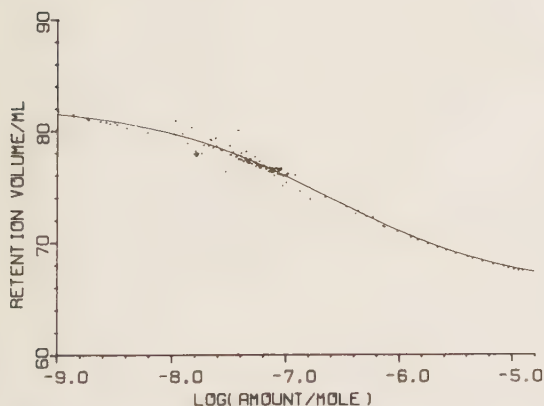


Fig. 1. Retention volume *versus* amount of sample for diisopropyl ether on 15% n -octadecane column.

The function

$$V_R = A + \frac{C}{\left(1 + B \cdot \sqrt{\frac{A_r}{V_R + V_g}} \right)^2} \quad (2)$$

has been fitted to the experimental points. A_r is the peak area, V_R the retention volume, V_g the void volume and A , B and C are constants.

Eqn. 2 was derived under the assumption that the retention volume is composed of contributions of bulk partition and adsorption at only one of the liquid interfaces. The first term, A , is equal to $K \cdot V_l$, where K is the partition coefficient, which, in the actual concentration range, can be considered to be constant, and V_l is the volume of the stationary phase. Thus, A can be interpreted as the contribution of bulk partition to the retention volume. The second term is derived from the Langmuir adsorption isotherm and expresses the influence of adsorption at different sample sizes. The constant C is the contribution from adsorption at infinite dilution ($A_r = 0$).

Eqn. 2 was found empirically to give a good representation of the experimental results for ethyl methyl ketone and diisopropyl ether on all of the *n*-octadecane columns. The theoretical consideration leading to this equation is beyond the scope of this paper and will be published elsewhere¹⁶.

The constants A , B and C can be determined by linear regression and simplex optimization¹⁷. The solid line in Fig. 1 was obtained with eqn. 2.

By inserting V_R from eqn. 2 into eqn. 1, the variation in Kováts retention index with sample size was calculated. Fig. 2 shows this variation for diisopropyl ether

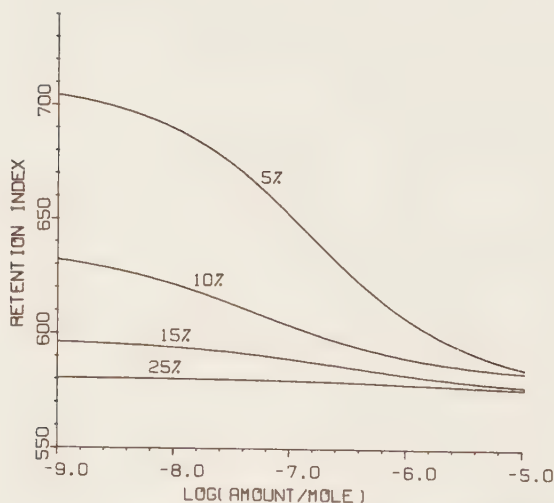


Fig. 2. Kováts retention index versus amount of sample for diisopropyl ether on *n*-octadecane columns.

and Fig. 3 for ethyl methyl ketone for the *n*-octadecane columns. It is apparent that the liquid phase loading strongly influences the Kováts retention index. The variation in retention index with stationary phase volume (V_L) and amounts of ethyl methyl ketone and diisopropyl ether injected is shown in Fig. 4. The upper curve for each solute corresponds to $A + C$ in eqn. 2 (infinite dilution) and the lower to A (large amounts injected). It is clear that the use of a high column loading and large amounts injected gives smaller variations in retention index. Hence the recommendations from several workers that samples as small as possible should be injected can be misleading^{1,18}.

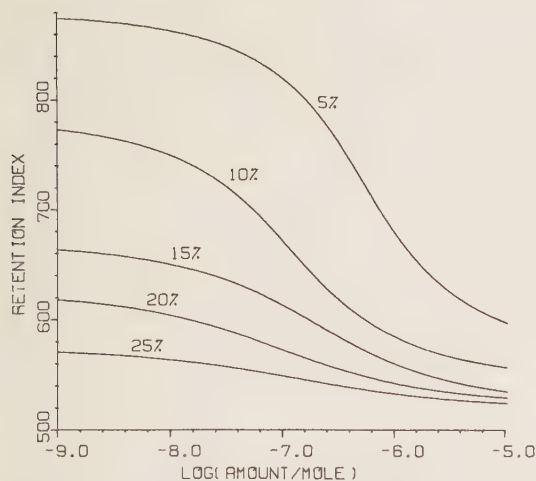


Fig. 3. Kováts retention index *versus* amount of sample for ethyl methyl ketone on *n*-octadecane columns.

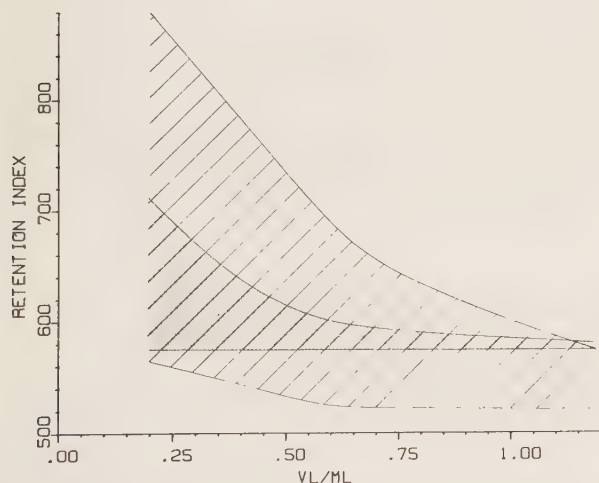


Fig. 4. Kováts retention index *versus* stationary liquid volume for diisopropyl ether (shaded) and ethyl methyl ketone (hatched) on *n*-octadecane columns.

Ethyl methyl ketone and ethyl acetate have been used as solutes in earlier investigations of complexation in binary stationary phase systems with a non-polar component (squalane) and a weakly polar component (dodecyl laurate or 1-chlorooctadecane)^{19,20}. In these systems a variation in retention volume with sample size was also found.

3,3'-Oxydipropionitrile (ODPN) column

Fig. 5 shows retention volume as a function of amount injected for some

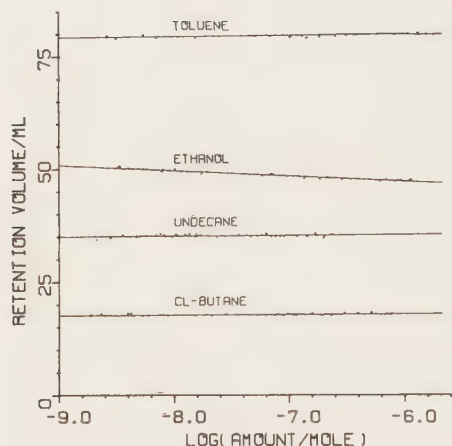


Fig. 5. Retention volume *versus* amount of sample for some solutes on 10% ODPN column.

solutes on an ODPN column with 10% (w/w) liquid phase loading. Similar plots were obtained on the other ODPN columns. Only for ethanol was the retention volume significantly influenced by the sample size.

When plots of retention volume *versus* liquid phase volume are made (Fig. 6), the regression lines for all solutes except the alkanes pass through the origin. The regression lines for the *n*-alkanes have significant positive intercepts. The retention volume for ethanol varies with sample size and therefore no definitive regression line can be drawn in this instance. This means that the adsorption effects for all solutes except the alkanes and to some extent ethanol are small on polar ODPN columns.

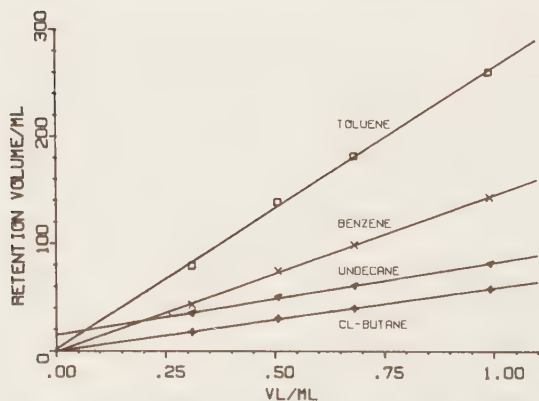


Fig. 6. Retention volume *versus* stationary liquid volume for some solutes on ODPN columns.

The contribution of adsorption to the retention volumes for *n*-nonane and *n*-undecane will greatly affect the Kováts retention index and the use of different liquid phase loadings leads to differences in retention index (see Fig. 7). The variations are smaller at high loadings. For ethanol, the variation in retention index with phase

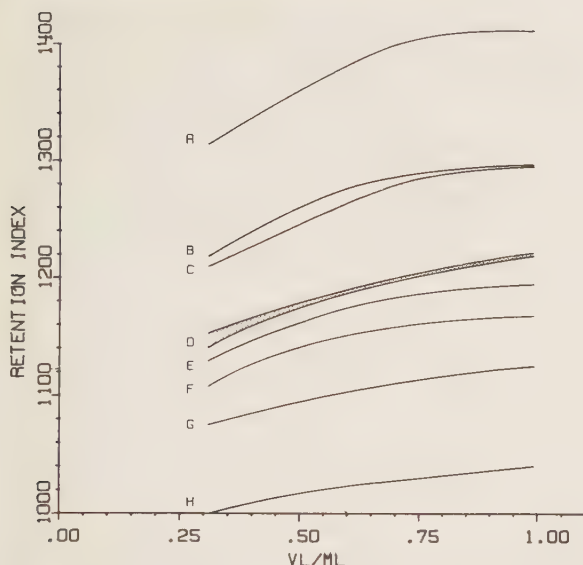


Fig. 7. Kováts retention index *versus* stationary liquid volume on ODPN columns. Solutes: A = acetonitrile; B = toluene; C = ethyl methyl ketone; D = ethanol; E = benzene; F = trifluorobenzene; G = di-*n*-butyl ether; H = 1-chlorobutane.

volume is described as an area between two curves. These curves give the limits of the variation of the retention index with amount injected, the upper curve corresponding to about $5 \cdot 10^{-9}$ mole and the lower curve to $5 \cdot 10^{-6}$ mole.

DISCUSSION

The results show that variations in Kováts retention indices, due to adsorption effects, are most pronounced on non-polar stationary phases with polar solutes. The Kováts retention index varies for polar solutes on non-polar columns because of adsorption contributions to the retention volume for the polar solutes. On polar columns, the variation in Kováts retention index is essentially due to adsorption effects for the alkane reference compounds.

For systems in which adsorption effects occur, it is obviously desirable to use conditions such that the adsorption is minimized and bulk partition is the main retention mechanism. For the *n*-octadecane system, eqn. 2 is in good agreement with the experimental points. The equation clearly shows that two measures can be taken in order to minimize the relative effect of the second term, which corresponds to adsorption effects. The first term can be increased by using a high liquid phase loading. The second term can be reduced by the injection of large samples, which means working in a range where the adsorption sites are saturated. Of course, the sample size must be small enough not to give appreciable effects on the partition constant due to changes in the bulk activity coefficients. In our experiments, an injection of about 10^{-6} mole per gram of stationary phase was found to be suitable. Figs. 2, 3 and 4 show clearly that it is advisable to use high liquid phase loadings and

large sample sizes. For the ODPN columns, the sample size is not important (Fig. 5) but here also high liquid phase loadings are desirable (Fig. 7).

It would be preferable if the same set of reference compounds could be used for several stationary phases. Instead of *n*-alkanes as reference compounds, homologous series of propyl ethers²¹, methyl esters²² and alkanols²³ have been suggested for polar stationary phases. Straight-chain aliphatic 2-ketones²⁴ have also been suggested as suitable for both polar and non-polar columns. Our experience suggests that *n*-alkanols are unsuitable on polar columns because of the variation in retention volume with amount injected and, for the same reason, 2-ketones are unsuitable on non-polar columns.

It would be interesting to try a set of alkylbenzenes as reference compounds, as they seem to behave almost ideally on several stationary phases of different polarities, *e.g.*, ODPN, alkyl ester¹⁹, phthalate esters²⁵, haloalkanes²⁰ and alkanes such as squalane¹⁹ and octadecane. Retention indices were calculated with eqns. 1 and 2 using benzene and toluene as reference compounds on the ODPN columns. For all solutes except the alkanes, the variation in retention index with stationary phase volume showed a 5–10-fold decrease compared with the Kováts retention indices. However, a variation of 5–10 retention index units still remains when the liquid phase loading is varied between 10 and 25% (w/w). These results again reflect the severe limitations of all retention index measurements. If the reference compounds and the other solutes investigated are not very similar with respect to functional groups, one can usually expect variations of all types of retention indices with liquid phase loading and with the amount injected.

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MIXED RETENTION MECHANISMS IN GAS-LIQUID CHROMATOGRAPHY

V. STUDIES OF ALKALI-TREATED CARBOWAX COLUMNS FOR AMINE ANALYSIS

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SUMMARY

The gas-liquid chromatographic analysis of free amines on Carbowax 20M in combination with KOH is investigated, especially with respect to the rôle of the alkali. The significance of the support properties (diatomite) and the influence of Carbowax 20M are also explored. The results show that a low alkali content (1 %) in combination with a high content (10 %) of the liquid loading is preferred. The negative effects of water as solvent are considerable for this column packing.

INTRODUCTION

Direct gas-liquid chromatography (GLC) of free amines leads to peak asymmetry, spurious peaks and insufficient reproducibility¹⁻⁵. These difficulties are magnified at low levels of amines in the samples.

Derivatization is often resorted to in order to overcome some of the difficulties^{6,7}. For aqueous samples, especially at low amine concentrations, most derivatization methods cannot be used. There are many environmental and hygienic samples for which adequate methods of analysis do not exist.

The above difficulties are caused by adsorption of the amines on the columns. Consequently, many different types of columns have been described⁸⁻¹⁰ where improvements in this respect have been attempted. The main strategy has been to create an alkaline environment in the column, to neutralize all acidic adsorption sites and to ensure that the amines exist in unprotonated form. This can be accomplished either by impregnating the packing with alkali^{2-4,11,12}, usually KOH, or by using a basic organic compound as stationary phase^{10,12,13} or as an additive^{1,10,13,14} ("tail reducer").

The introduction of nitrogen-selective detectors based on ceramic beads, *e.g.*, Varian's thermionic specific detector, has improved the detection of amines in the presence of an excess of nitrogen-free organic compounds. To utilize the advantages of this detector, alternatives must be found to nitrogen-containing organic column materials, since the inevitable bleeding will result in baseline drift. It should be poin-

ted out that all alkaline organic compounds used so far contain nitrogen. The only GLC columns of practical interest, for use with nitrogen-selective detectors, are those containing alkali. With these columns, however, problems also arise due to decomposition of some substances at increased column temperatures, especially when water is employed as solvent¹⁵.

This paper reports on the rôle of alkali in a model system. The properties of the support and of the stationary phase are also investigated. The combination of Carbowax 20M (CW) and KOH was chosen, since this is one of the most commonly used for amine analysis. Another stationary phase which has been used in combination with KOH, in most cases with better results, is Pennwalt 223¹⁶. However, this compound is not sold separately and its chemical formula is patented.

EXPERIMENTAL

Instruments

Retention volumes were measured and processed with the GC equipment described elsewhere^{17,18}, which enables direct measurements of the retention volume. Experience from studies of GC retention mechanisms¹⁹ formed the basis for the interpretation of the results. Vapour samples of widely varying concentrations were injected under computer control and the retention volumes were measured at the maximum of the peaks. To elucidate the influence of the silanization of supports, some experiments were performed with a Varian 2700 gas chromatograph with a flame-ionization detector (FID). The effect of water as solvent was studied with a Varian 3700 with a thermal conductivity detector (TCD).

Columns

Chromosorb W AW (Johns-Manville, Denver, CO, U.S.A.) was used as support. The packings for the precision gas chromatograph were made with 45–60 mesh support and for the other gas chromatographs 100–120 mesh support was used. Some experiments were conducted with silanized support (Chromosorb W AW DMCS).

The packing was prepared according to Conder and Young²⁰. Carbowax 20M (CW) (Supelco, Bellefonte, PA, U.S.A.) and KOH (87.9%; EKA, Bohus, Sweden) were dissolved in methanol (p.a., E. Merck, Darmstadt, G.F.R.). Dead volumes were measured by the injection of methane.

RESULTS AND DISCUSSION

Unsilanized supports

Variation of KOH. Retention volumes for triethylamine, piperidine and hexylamine were measured over a wide range of sample sizes, on columns with 10% CW and various concentrations of KOH (0–5%) on unsilanized support (Chromosorb W, AW). In Fig. 1 the results for piperidine are shown. Similar curves were obtained for triethylamine and hexylamine with the exception that no chromatograms can be obtained for hexylamine on the column without KOH.

The retention volume increases as the amount of sample decreases, quite drastically at low alkali contents of the columns. With increasing sample size all the curves seem to converge towards a common retention volume, *i.e.*, independent of the alkali

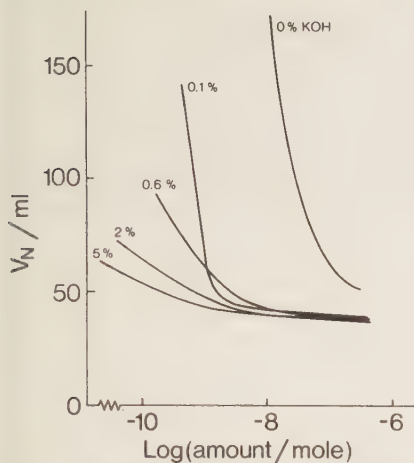


Fig. 1. Net retention volumes of piperidine vs. the logarithm of the amount of sample on columns with 10% Carbowax 20M and different contents of KOH. Glass columns, V-shaped (80 cm $\times$ 4 mm I.D.), were used at 80°C with H_2 as carrier gas (60 ml/min) and FID.

concentration. With a large sample all adsorption sites become saturated with only a small fraction of the sample. Most of the sample is then retained on the column by a partition equilibrium. With small samples, on the other hand, the retardation is mostly caused by adsorption. Since adsorption causes a stronger retardation than partition, the retention volume will increase. Both partition and adsorption contribute to the observed behaviour.

In addition, it can be seen that the adsorption decreases as the alkali content increases and that this dependence is especially strong at very low alkali contents. The effect diminishes on further addition of alkali above 2–5% KOH. Still, at 5% KOH the retention volume varies by 40% over the sample size range.

Fig. 2 shows the peak shape on a column with no KOH and on a column with high KOH content. The peaks are skewed as expected for retention caused by both adsorption and partition.

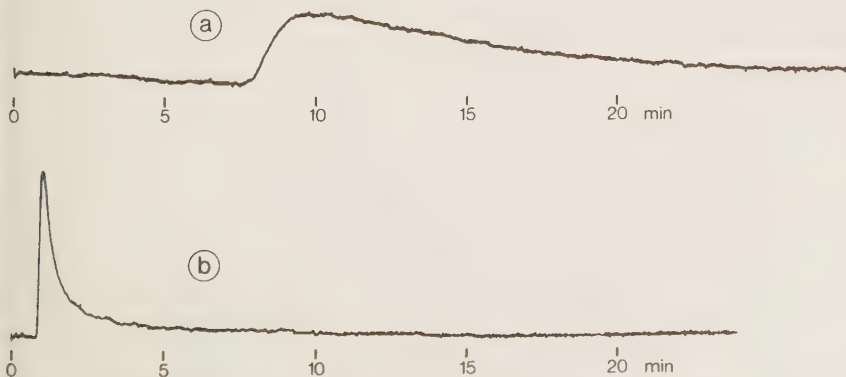


Fig. 2. Chromatograms of about $2 \cdot 10^{-9}$ moles of piperidine on columns with 10% Carbowax 20M and 0% KOH (a) and 10% Carbowax 20M and 5% KOH (b). Detector sensitivity 2×10^{-12} A. Other conditions as in Fig. 1.

Variation of CW. The variation of the retention volume with sample size was investigated for various CW contents in the range 1–15%. The KOH content was kept constant at 1%. The result for piperidine is shown in Fig. 3. Analogous results were obtained for triethylamine and hexylamine. The curves are very similar, but the retention volume increases with liquid loading. This indicates that the adsorption effects are similar, but that the partition effect varies. We find that the retention volume is proportional to the amount of CW in the columns at large sample sizes.

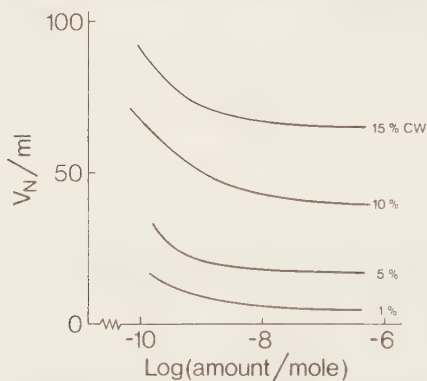


Fig. 3. Net retention volumes of piperidine vs. the logarithm of the amount of sample on columns with 1% KOH and different percentages of Carbowax 20M. Other conditions as in Fig. 1.

We conclude that for the amines the partition depends on the CW amount and that the adsorption effects depend on the KOH concentration. Thus, for small adsorption effects relative to partition, we should have at least 1–2% KOH and a high percentage of CW. To illustrate this point, in Fig. 4 we compare chromatograms of some homologous amines on two columns, both with 1% KOH, but with different CW loading. The tailing is comparable, but the separation increases with increasing CW loading.

Silanized vs. unsilanized supports

With silanized supports the tailing of the amine peaks is more pronounced than with unsilanized supports, see Fig. 5. There is also an increase in retention time. Both

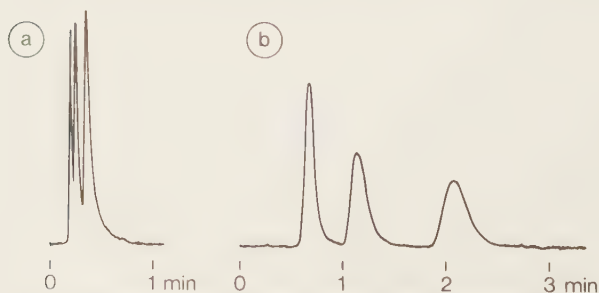


Fig. 4. Chromatograms of butyl-, pentyl- and hexylamine on columns with 1% Carbowax 20M and 1% KOH (a) and 15% Carbowax 20M and 1% KOH (b). Detector sensitivity 2×10^{-9} A. Other conditions as in Fig. 1.

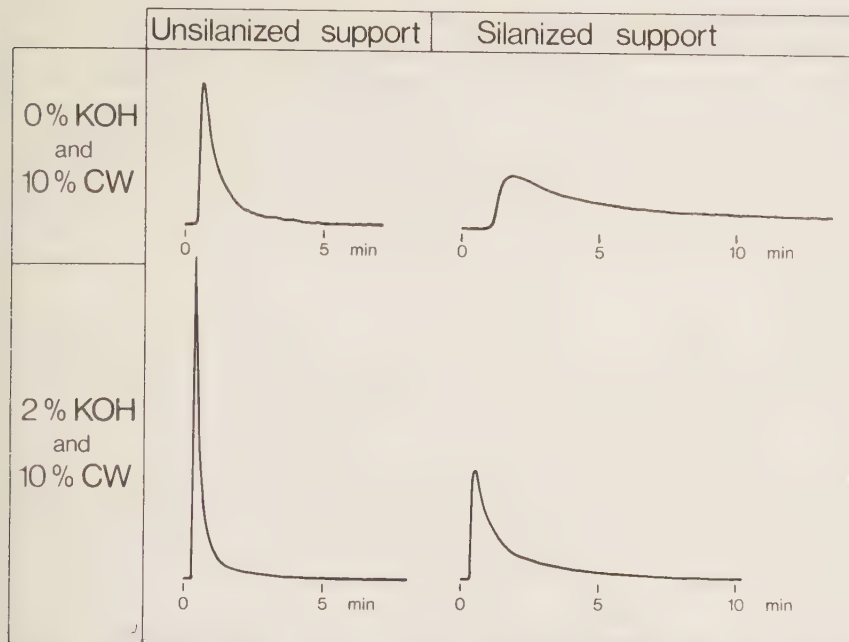


Fig. 5. Comparison of chromatograms of triethylamine on columns with 10% Carbowax 20M, 0% KOH or 2% KOH on silanized and unsilanized supports. Glass columns ($2 \text{ m} \times 2 \text{ mm I.D.}$) were used at 80°C with N_2 as carrier gas (25 ml/min) and with a FID sensitivity of $4 \times 10^{-12} \text{ A}$.

these observations demonstrate that the adsorption effects are larger on silanized than on unsilanized supports.

Unsilanized supports, which have a high capacity for hydrogen bonding²¹, are completely wetted by CW (*cf.*, the use of CW for support deactivation^{22,23}). This fact, together with the use of a relatively high liquid loading, results in filling of pores and cavities, creating a low surface area of the stationary phase (the gas-liquid interface).

With a silanized support the weakly acidic, strongly adsorbing, silanol groups are replaced with the reagent. On this non-polar surface, the wetting is not complete, so the stationary phase will form an inhomogeneous layer as droplets, resulting in a larger surface area of the stationary phase¹⁹. The increased tailing of the amine peaks with silanized supports results from both the increased adsorption due to the larger surface area of the stationary phase and the adsorption effect due to interaction between the polar amines and the non-polar silanized support surface, partly free of liquid loading (*cf.*, the opposite polarity situation in ref. 19). Further evidence is the irreversible adsorption of the amines on the uncoated unsilanized support. On an uncoated silanized support the sample passes through and is retarded by adsorption, due to the same polar-non-polar interaction as above, resulting in extensive tailing.

An unsilanized silica surface contains a large number of silanol groups. However, the amount of KOH required to neutralize these groups is very small. As shown in Fig. 1, the KOH concentration is of importance in achieving less tailing of amine peaks, and should be far above the level necessary for the neutralization. If the

concentration of surface hydroxyl groups is $2.5 \cdot 10^{19}$ groups per m^2 (ref. 24), a KOH concentration of 2% is ten times higher than necessary for the neutralization.

Thus, it is important to add alkali to reduce the adsorption effects, but it should be noted that the nature of the support surface is even more important than the addition of KOH, see Fig. 5. It should be mentioned that one (out of four) batch of Chromosorb W AW gave surprisingly severe tailing and could not be used for amine analysis. This was probably due to insufficient rinsing in the factory after the acid washing.

Water as solvent

All samples reported so far were injected directly as vapours, *i.e.*, without solvent. Aqueous samples are of importance in many applications. To study the behaviour of water as solvent, injections of 1–5 μl water were made on columns having different contents of KOH and CW, within the temperature interval 80–170°C (thermal conductivity detector).

The chromatograms in Fig. 6 show that water gives a severely tailing peak in the essential part of the chromatogram, in accordance with previous results^{8,11,16}. The peak width increases with the percentage of KOH in the packing. The water is strongly adsorbed and its retardation is approximately proportional to the amount of KOH in the columns. The amount of CW is of much less importance for the retention and peak shape of water. Finally, the sensitivity of the detector is also reduced when the water elutes.

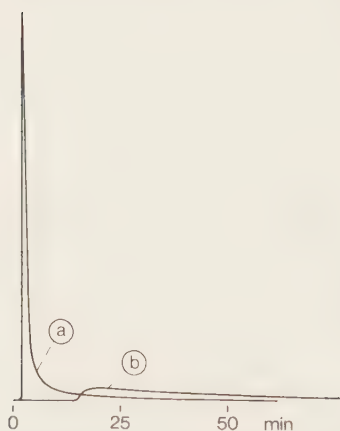


Fig. 6. Chromatograms of 5 μl water on columns with 10% Carbowax 20M and 0.5% KOH (a) and 10% Carbowax 20M and 5% KOH (b). Glass columns (2 m $\times$ 4 mm I.D.) were used at 110°C with N_2 as carrier gas and TCD.

CONCLUSIONS

Addition of KOH to the columns strongly decreases the adsorption of amines. However, an adverse effect of the KOH addition is a strong retardation of the water peak. The amount of Carbowax 20M of course influences the retention of amines, but has little effect on the adsorption. Furthermore, the effect of CW loading on the retention of water is negligible compared with the influence of KOH.

For amine analysis, addition of alkali is necessary for CW columns. It is advisable to use a relatively high loading of CW and the support should not be silanized. Suitable parameters are 10% (w/w) CW and 1% KOH on Chromosorb W AW. Such a column is very useful for samples in non-aqueous solvents, but water will interfere with the analysis.

It is clear that the combination of CW and KOH is not a perfect system for amine analysis. Further research directed towards basic modification of supports, the use of very inert supports and the use of basic stationary liquids may result in better columns for amine analysis without the negative effects of KOH treatments.

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A Static Technique for the Determination of Partition Coefficients by Gas Chromatography

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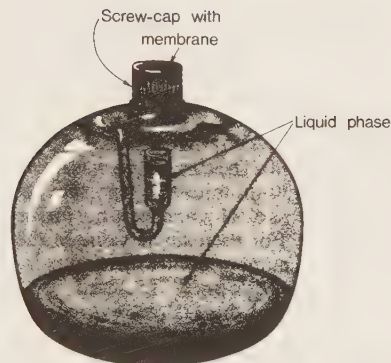


Fig. 1. Equilibrium vessel for the determination of the partition coefficient.

Studies of the solubility of volatile compounds in solvents of high molecular weight are of interest from two points of view. They contribute to the knowledge of the thermodynamics of solutions and they are important in establishing the validity of the theory of gas-liquid chromatography.

A static technique based on measurements in equilibrated heterogeneous systems of the partition coefficient, K , between liquid phase and gas phase has been developed. In this technique the adsorption of the solute on the solid support surface or liquid surface are negligible in contrast to measurements with the GLC-technique. In a number of papers^{1–7} versions of the static method are described. In all but one⁷ the determination of K is reduced to a gas chromatographic analysis of the gas phase before and after equilibration with the liquid phase. In these methods the desorption of the solute molecules from the walls of the vessel, when the liquid phase is added, introduces an additional source of error. Berezkin *et al.*⁷ performed the analysis on both phases after equilibration, and reached a coefficient of variation of 3.5%. A similar technique was used in this work but with better standardization of the experimental conditions, the precision was significantly improved.

Experimental. Apparatus. The vessel in which the equilibrium was attained (Fig. 1), is a 1000 ml glass bottle with a teflon-lined septum. Inside the bottle a small open container was positioned a few centimeters below the septum so that liquid samples could be taken with a micro-syringe with a needle length of 5 cm. The small container was fixed to a bent glass rod which was fused to the vessel. A part of the liquid was also placed as a thin layer at the bottom of the vessel in order to facilitate equilibration. The large volume of the vessel was chosen in order to minimize the reduction of the pressure when the gas phase is sampled.

The vessel was loaded with about 15 ml of the liquid phase and flushed with dry nitrogen before it was closed. The solute was added via the teflon-lined septum with a micro-syringe (less than 30 μ l). The partition coefficient is a constant only when Henry's law is valid. The linear region is observed at concentrations less than 0.3% according to Berezkin *et al.* The present work has been performed at solute concentrations in the region of 0.02–0.2%. The vessel was immersed in a thermostatically controlled waterbath and equilibrium was attained after about 24 h. This slow approach to equilibrium depends not only on the slow diffusion in the liquid phase but also on the time needed for obtaining homogeneous temperature in a litre of gas. The time can be reduced with an effective stirring system. The pressure of the vessel was adjusted to the ambient with dry nitrogen via a needle through the septum.

The concentration of the solute in the vapour and liquid phases was measured with a Varian Gas Chromatograph, model 3700, with FID. The peak areas in the chromatogram were determined with a Varian digital integrator, model 481. One of the injection ports was exchanged for a six-port gas sampling valve mounted in the column oven. The volume of the gas sampling loop was 0.262 ± 0.002 ml with 95% confidence and it was determined by filling it with a lead nitrate solution which was titrated with EDTA.

To ensure that the valve was filled with a homogeneous gas sample, it was flushed with 5 ml of the sample gas. A waiting time of 10 s before injection was found to be appropriate to obtain temperature and pressure equilibrium. The temperature of the column oven, and thus the sample loop, was kept approximately the same as for the equilibrium vessel. Two columns were used, one connected to the six-port valve for the measurements of the gas phase, and the other connected to a conventional injection port for the measurements of the liquid phase. Both columns were connected to the same FID to ensure the same sensitivity. The columns were of stainless steel (2 m long, 2 mm i.d.) packed with 60–80 mesh Chromosorb AW-HMDS coated with 15% Carbowax 1540. The carrier gas was nitrogen with a flow-rate of 30 ml/min. All chemicals had a stated purity in excess of 99% and were not further purified.

Procedure. For each single value of the partition coefficient a number of measurements in the liquid phase was performed followed by a single determination in the gas phase. Immediately after, the pressure in the vessel was equilibrated. It was sufficient to wait 30 min between each run to obtain partition equilibrium. Differences in temperature or pressure between the vessel and the gas sample valve result either in expansion or concentration of the gas volume. To be able to relate the peak area of the solute to the concentration of the solute in the vessel, temperature and pressure of the system were strictly controlled. The temperature was measured with mercury thermometers (0.05 °C/div.) with a maximum variation of 0.1 °C and calibration traceable to NBS standards; the pressures were measured with a mercury manometer read to a precision of 0.05 mmHg.

If the gas phase is considered as ideal the solute concentration (C_g) in the equilibrium vessel is given by

$$C_g = (P_g/RT_g)x \quad (1)$$

and the injected solute concentration (C_i) by

$$C_i = (P_i/RT_i)x \quad (2)$$

P_g , T_g and P_i , T_i refer to the pressure and temperature of the equilibrium vessel and gas sampling valve, respectively. x is the molar fraction of the solute and R is the gas constant. Hence

$$C_g = (T_i P_g C_i)/(P_i T_g) \quad (3)$$

The partition coefficient is calculated from

$$K = C_l/C_g = (A_l V_g T_g P_i)/(A_g V_l P_g T_i) \quad (4)$$

A_l , V_l and A_g , V_g refer to the areas of the solute peaks and the injected volumes of the liquid and gas phases, respectively.

Results and discussion. The method was applied for n-pentane, n-heptane, diisopropyl ether and ethyl methyl ketone as solutes at 60 °C in n-octadecane as solvent. The results are summarized in Table 1. These systems were chosen in order to check the validity of a new model by Jönsson and Mathiasson⁸ for calculating the partition coefficient from gas chromatographic retention data measured in the presence of strong surface adsorption effects. In experiments 1 and 2, two solutes were run simultaneously. Good agreement was obtained whether the experiments were conducted with a single solute (see Table 1; 3–5) or in a mixture (see Table 1; 1, 2). Provided that the total amount of solute molecules in the mixture does not exceed the region where Henry's law is valid, the partition coefficient of the volatile components of a multicomponent system can be determined.

In Table 2 the weighted means from different experiments for each solute are compared with the corresponding values of the partition coefficient given by Jönsson and Mathiasson.⁸ Good agreement is obtained.

Table 1. Gas-liquid partition coefficients with n-octadecane as solvent at 60.0 °C. Values are given with 95% confidence intervals.

Solute	Exp.	K at 60.0 °C	Number of measurements
n-Pentane	1	47.5 ± 0.2	19
n-Pentane	2	47.8 ± 0.3	28
n-Heptane	2	325 ± 2	28
Ethyl methyl ketone	3	59.5 ± 0.4	7
Ethyl methyl ketone	4	59.2 ± 0.7	26
Diisopropyl ether	1	99.6 ± 0.4	19
Diisopropyl ether	5	99.2 ± 0.3	26

Table 2. Comparison of gas-liquid partition coefficients determined by the present static technique and from gas chromatographic retention data. Solvent, n-octadecane. Values are given with 95 % confidence intervals.

Solute	K at 60.0 °C	
	This work	Ref. (8)
n-Pentane	47.7 ± 0.2	48.2 ± 3.9
n-Heptane	325 ± 2	—
Ethyl methyl ketone	59.2 ± 0.6	58.8 ± 3.5
Diisopropyl ether	99.4 ± 0.3	101.4 ± 3.9

The precision of the static method is strongly influenced by the manner in which the liquid phase and especially the gaseous phase are withdrawn and injected in the gas chromatograph. The use of n-octadecane as solvent requires, however, some comment. With repeated injections the column changes slowly but continuously, as the n-octadecane is not eluted, resulting in wider peaks and base line changes and it has to be replaced after about one hundred injections. Due to the high melting point of the solvent, the temperature of the injection syringe has to be maintained above 28.2 °C. However, under these conditions the accuracy and precision of the injected volume of the liquid phase is improved compared with injections of the volatile solvents, normally used. Furthermore, the use of a gas loop injection valve is indispensable. A standard gas tight injection syringe, at least when not completely new, does not guarantee good reproducibility of the volume injected.

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DETERMINATION OF THE LOSS OF STATIONARY PHASE IN GAS-LIQUID CHROMATOGRAPHY FROM THE CHANGE IN RETENTION VOLUME

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SUMMARY

The effects of liquid-phase bleeding on the determination of gas chromatographic quantities are important, especially when using a high-precision gas chromatograph. Equations are developed in which changes in solute retention volume are used to correct for losses of stationary phase. The method gives the best precision when frequent measurements are made of solute retention volume and of the corresponding total volume of carrier gas that has passed through the column, and is therefore most suited to chromatographs coupled to an on-line computer. It has been found that the change in solute retention volume with accumulated carrier gas flow is constant with *n*-octadecane and 3,3'-oxydipropionitrile as stationary phases; no influence of flow-rate has been observed in the range 25-85 ml/min. Pressures of octadecane at 333 and 371 °K, and of 3,3'-oxydipropionitrile at 373 °K, have been calculated from the bleeding. The values obtained agree well with literature values for the saturated vapour pressure. Thus, in many cases, the change in volume of the stationary phase with accumulated flow can be calculated from the saturation vapour pressure.

INTRODUCTION

If the bleeding of the stationary phase is ignored during extended use of a column, then an error due to the change in the stationary phase volume is introduced into the calculations of partition coefficients. When using a high-precision gas chromatograph this error can be of the same magnitude as the sum of all of the other errors in the measurements. Hence there is a need to know the correct stationary phase volume at the time of an experiment. The possibility of using the retention volume of the solute to correct for weight losses from the stationary phase in gas-liquid chromatography (GLC) was shown by Keller *et al.*^{1,2}. Later Berezkin *et al.*³ studied the thermal stability of stationary liquid phases in a similar way, by assuming partition as the only mechanism responsible for solute retention. In the theoretical section below, equations are developed in which measured changes in the solute retention volume can be used to correct for losses in stationary phase at any time

during the lifetime of a column. When the carrier gas is saturated with the vapour of the stationary phase, the equations can also be used to determine the vapour pressure of the organic compound used as the stationary phase.

THEORY

The retention volume, V_N , corrected for pressure drop for a solute on a stationary phase when partition is accompanied by adsorption phenomena can be written, according to Conder *et al.*⁴ and Suprynowicz *et al.*⁵, as

$$V_N = V_l K_l + V_s K_s + V_i K_i \quad (1)$$

where K_l , K_s and K_i are the partition coefficients for bulk liquid solution, support surface adsorption and liquid surface adsorption, respectively, V_s and V_l are volume expressions for the surface zones in which the support surface and liquid surface adsorption occur and V_i is the volume of the stationary phase. During column use, V_l decreases because of bleeding of the stationary phase. If the change in V_l with the accumulated carrier gas flow is constant, then the volume of the stationary phase at any accumulated flow is given by

$$V_l = V_l^B + \frac{dV_l}{dV_{acc}} \cdot V_{acc} \quad (2)$$

where V_{acc} is the total volume of carrier gas that has passed through the column since it was inserted into the chromatograph and V_l^B is the volume of the stationary phase when V_{acc} is zero.

The terms $V_s K_s$ and $V_i K_i$ in eqn. 1 can be considered as constants, provided the loading is relatively high, so that V_s and V_i are approximately constant. Then eqn. 1 can be written as

$$V_N = V_l K_l + A \quad (3)$$

where $A = V_s K_s + V_i K_i = \text{a constant}$. Differentiating eqn. 3 with respect to V_l gives:

$$\frac{dV_N}{dV_l} = K_l \quad (4)$$

The multiplication of differentials

$$\frac{dV_N}{dV_l} \cdot \frac{dV_l}{dV_{acc}} = \frac{dV_N}{dV_{acc}} \quad (5)$$

and eqns. 2 and 4 give:

$$V_l = V_l^B + \frac{dV_N}{dV_{acc}} \cdot \frac{1}{K_l} \cdot V_{acc} \quad (6)$$

In eqn. 6, V_1 is a linear function of the total accumulated carrier gas volume, V_{acc} , provided dV_N/dV_{acc} is constant. When V_1^B , dV_N/dV_{acc} and K_1 for a solute have been determined for a given stationary phase at a certain temperature, a true value of V_1 can be calculated at any accumulated carrier gas volume. V_1^B can be determined at the preparation of the column, and dV_N/dV_{acc} can be obtained as described in the Experimental section. Depending on whether or not adsorption occurs, K_1 can be determined in two slightly different ways.

No adsorption occurs

Here $V_N = V_1 K_1$. For the determination of K_1 the volume of the stationary phase and the corresponding retention volume are needed. It is best to use the stationary phase volume V_1^B measured when the column is prepared because it is easy to determine. Thus, if V_N^B is the retention volume when V_{acc} is zero, then:

$$K_1 = V_N^B / V_1^B \quad (7)$$

If the retention volume V_N^E at the end of the experiment and the corresponding stationary phase volume V_1^E are used:

$$K_1 = V_N^E / V_1^E \quad (8)$$

Adsorption occurs

Here eqn. 3 is applicable. In this case we must know the stationary phase volumes and the corresponding retention volumes at two different occasions, preferably at the beginning and at the end of the experiment. Then eqn. 3 gives

$$V_N^B = V_1^B K_1 + A$$

$$V_N^E = V_1^E K_1 + A$$

leading to:

$$K_1 = \frac{V_N^B - V_N^E}{V_1^B - V_1^E} \quad (9)$$

Thus eqn. 6 combined with 7 (no adsorption) or 9 (adsorption) can be used to calculate the volume of the stationary phase at any value of the accumulated flow. The slope dV_N/dV_{acc} is determined from retention-volume measurements as described in the Experimental section.

Simplified method

The slope in eqn. 6 can also be calculated from the saturation vapour pressure of the stationary phase, provided the carrier gas flow-rate is sufficiently low for the carrier gas to be saturated with the vapour of the stationary phase at the column outlet. Then

$$n = \frac{p \cdot V_{acc}}{RT} \quad (10)$$

where n is the number of moles of stationary phase that have evaporated, p is the saturation vapour pressure of the stationary phase at the temperature T and R is the gas constant. Differentiating n with respect to V_{acc} gives:

$$\frac{dn}{dV_{acc}} = \frac{p}{RT} \quad (11)$$

The change in the number of moles in the liquid stationary phase given by $(dV_l/dV_{acc}) \cdot (\delta/M)$ is equal but of opposite sign to the change in the number of moles in the vapour phase given by dn/dV_{acc} . Thus

$$\frac{dV_l}{dV_{acc}} = -\frac{dn}{dV_{acc}} \cdot \frac{M}{\delta} \quad (12)$$

where M is the molecular weight and δ is the density of the stationary phase.

Eqns. 4, 5, 11 and 12 give:

$$\frac{dV_N}{dV_{acc}} \cdot \frac{1}{K_1} = -p \cdot \frac{M}{RT\delta} \quad (13)$$

If p and δ are known, $(dV_N/dV_{acc}) \cdot (1/K_1)$ may be obtained from eqn. 13 and used in eqn. 6 to correct for column bleeding. Unfortunately, very few values of p are available for different stationary phases.

Calculation of stationary phase pressure

With $V_1^B = w_1^B/\delta$ where w_1^B is the weight of the stationary phase when the column is packed, eqns. 7 and 13 give:

$$p = -\frac{dV_N}{dV_{acc}} \cdot \frac{w_1^B}{V_N^B} \cdot \frac{RT}{M} \quad (14)$$

Thus when dV_N/dV_{acc} is determined from retention-volume measurements, the stationary phase pressure can be calculated. Since there is a choice in selecting the solute, it is usually not difficult to find a solute for which adsorption effects can be neglected and to use eqn. 14 for the calculations of dV_N/dV_{acc} or p .

EXPERIMENTAL

All of the measurements were made by use of a high-precision gas chromatograph coupled on-line with an Alpha LSI-2 mini-computer, with 16 K of core memory. This system, developed by Jönsson and co-workers^{6,7}, is described elsewhere.

Two stationary phases, *n*-octadecane ($n\text{-C}_{18}$) and 3,3'-oxydipropionitrile (ODPN) were used in our investigation of column bleeding in GLC. Seven V-shaped columns (1000×4 mm I.D.) were used, containing *ca.* 4 g of packing loaded with *ca.* 20% w/w of stationary phase. Supasorb (40–60 mesh), acid washed and treated with hexamethyldisilazane (BDH, Poole, Great Britain), was used as the support. Hydrogen was the carrier gas and methane was used for the determination of the


```

ODPN-1          BUTHYL ACETATE          75 06 24    BLEEDING

BASELINE NOISE   181    186

RUN NO. 45

TEMPERATURE 99.565  99.5559

MINUTES 431      ACC. FLOW 24694.9
P 760.021        DP 81.2059

VR              AREA              VN              1/N              SKEW
5.04848         4.60898              0.183143      0.292076
69.4993         3.85251              64.4509       4.14967E-03   0.698469
TAPE 7 ADDRESS 1614

```

Fig. 1. Computer output for one run. The list shows the name of the column and sample, the date and type of experiment, values signifying the baseline noise, the temperature before and after the run, the time since the start of the experiment, the volume of carrier gas that has passed through the column and the outlet pressure (P) and the pressure drop (DP) along the column. The following table gives the retention volumes V_R and V_N , where V_N is corrected for the void volume, the area and the skew of the peaks and $1/N$ where N is the number of theoretical plates.

void volume. Vapour samples, mixtures of methane and other solutes, were automatically injected during each experiment. After each run, completed in 10–20 min, the computer registered a number of parameter values which were recorded on a cassette tape and printed on a teletypewriter. A typical list produced after a run can be seen in Fig. 1.

The retention volume was calculated by the computer using the method of statistical moments⁸. The volume of carrier gas that has passed through the column, corrected to 1 atm pressure and column temperature, is based on measurements of the flow-rate made each minute. A typical experiment required *ca.* 40 h. Other essential experimental conditions are given in Table I.

The amount of packing material and the percentage of the stationary phase was carefully measured at the preparation of the columns. For columns 1 and 5, the remaining percentage of stationary phase after completion of an experiment was also determined by means of a combustion procedure described elsewhere⁹. When an experiment had been completed, the computer was used to calculate the regression lines.

TABLE I

EXPERIMENTAL CONDITIONS FOR THE BLEEDING EXPERIMENTS

a = *n*-heptane, b = benzene, c = *n*-nonane, d = butyl acetate.

Column	Stationary phase	Solute	Temp. (°K)	Flow (ml/min)	Accumulated flow, V_{acc}^E (l)	No. of injections
1	<i>n</i> -C ₁₈	a, b	333.20	26.2	75.4	92
2	<i>n</i> -C ₁₈	a, b	333.16	41.4	106.1	152
3	<i>n</i> -C ₁₈	a, b	333.20	51.1	98.4	83
4	<i>n</i> -C ₁₈	a, b	333.17	62.1	111.1	115
5	<i>n</i> -C ₁₈	a, b	333.22	84.1	64.2	64
6	<i>n</i> -C ₁₈	c	370.85	61.4	218.3	177
7	ODPN	d	372.68	58.0	157.3	289

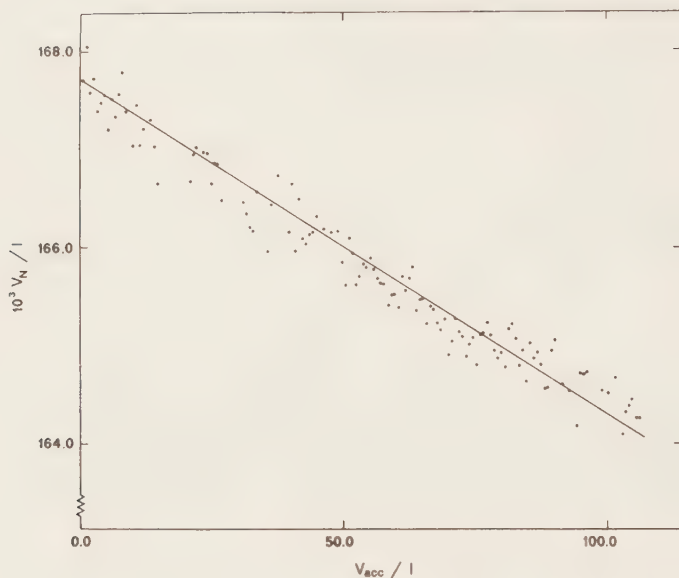


Fig. 2. Plot of the retention volume for benzene *versus* the total accumulated carrier-gas volume corrected to column temperature and 1 atm pressure for column 2.

RESULTS AND DISCUSSION

The retention volume for the solutes decreased linearly when plotted *versus* the total volume of carrier gas that had passed through the column (Fig. 2). Eqn. 6 can then be used to correct for the change in V_1 on both the non-polar octadecane and the rather strongly polar ODPN columns. Thus the change in retention volume can be used to correct for changes in the volumes of the stationary phases independently of the polarity of the stationary phases.

The changes in solute retention volumes with accumulated carrier gas volumes for the different columns were calculated from the regression lines which were based on the number of experimental points given in Table I. The solute retention volumes at the beginning of the experiments were calculated from the regression lines by inserting $V_{acc} = 0$. Values of dV_N/dV_{acc} and V_N^B together with their 95% confidence intervals are given in Table II. A thermogravimetric investigation¹⁰ has shown that a maximum systematic error of 0.1% can arise in the determination of phase loading due to the adsorption of water on the support. The resulting systematic error in the determination of phase bleeding or of stationary phase pressure is negligible and has not been taken into account. Densities used in the calculation of V_1 for octadecane and ODPN were calculated from data in ref. 11 and refs. 12 and 13, respectively. The values used for octadecane were 0.7558 g/cm³ at 333.2 °K and 0.7300 g/cm³ at 370.9 °K, and for ODPN, 0.979 g/cm³ at 372.7 °K.

K_1 and p were calculated from eqns. 7 and 14 in which adsorption effects were not considered. The adsorption effects are negligible for solutes such as *n*-heptane and benzene on columns loaded with long-chain alkanes, as they are expected to dissolve easily in the stationary phase. This has been discussed in a previous paper⁹. In the

TABLE II

RESULTS

For a-d see Table I. Values are given with 95% confidence intervals.

Column	Solute	Weight of stationary phase, $V_1^B(g)$	Retention volume, $V_N^B (ml)$	Change of retention volume, $10^5 \cdot \frac{dV_N}{dV_{acc}}$	Partition coefficient, $K_1 = \frac{V_N^B}{V_1^B}$	$10^7 \cdot \frac{dV_N}{dV_{acc}} \cdot \frac{1}{K_1}$	Pressure, $p (Pa)$
a		0.75592	332.5 $\pm$ 0.4	6.62 $\pm$ 0.80	332.4 $\pm$ 0.4	1.99 $\pm$ 0.24	1.44 $\pm$ 0.17
b			177.8 $\pm$ 0.3	4.10 $\pm$ 0.46	177.8 $\pm$ 0.3	2.30 $\pm$ 0.26	1.67 $\pm$ 0.19
a		0.74030	313.7 $\pm$ 0.2	5.44 $\pm$ 0.30	320.3 $\pm$ 0.2	1.70 $\pm$ 0.09	1.23 $\pm$ 0.07
b			167.9 $\pm$ 0.1	3.43 $\pm$ 0.18	171.4 $\pm$ 0.1	2.00 $\pm$ 0.11	1.45 $\pm$ 0.08
a		0.82810	364.0 $\pm$ 0.2	7.80 $\pm$ 0.36	332.2 $\pm$ 0.2	2.35 $\pm$ 0.11	1.70 $\pm$ 0.08
b			194.6 $\pm$ 0.1	4.55 $\pm$ 0.12	177.6 $\pm$ 0.1	2.56 $\pm$ 0.07	1.86 $\pm$ 0.05
a		0.72464	306.2 $\pm$ 0.1	4.27 $\pm$ 0.27	319.4 $\pm$ 0.2	1.34 $\pm$ 0.08	0.97 $\pm$ 0.06
b			163.6 $\pm$ 0.1	2.39 $\pm$ 0.11	170.7 $\pm$ 0.1	1.40 $\pm$ 0.06	1.02 $\pm$ 0.05
a		0.76296	328.0 $\pm$ 0.3	5.02 $\pm$ 0.79	324.9 $\pm$ 0.3	1.55 $\pm$ 0.24	1.12 $\pm$ 0.18
b			175.3 $\pm$ 0.1	3.11 $\pm$ 0.30	173.6 $\pm$ 0.1	1.79 $\pm$ 0.17	1.30 $\pm$ 0.12
c		0.64785	188.9 $\pm$ 0.1	68.54 $\pm$ 0.08	212.8 $\pm$ 0.1	32.21 $\pm$ 0.04	32.92 $\pm$ 0.04
d		0.64648	67.87 $\pm$ 0.05	14.09 $\pm$ 0.08	102.8 $\pm$ 0.1	13.71 $\pm$ 0.07	26.4 $\pm$ 0.1

same way, a polar solute such as butyl acetate is expected to dissolve in the polar ODPN phase with negligible adsorption effects. The very small adsorption effects in these cases is further confirmed by the low skew measured on both of the octadecane and ODPN columns.

A comparison between values of p (Table II) for octadecane columns, run with different flow-rates (Table I), shows no marked dependence on flow-rate. Turkel'taub and Luskina¹⁴ found, using a capillary column (1 m $\times$ 1 mm I.D.), that the pressure of $n\text{-C}_{13}\text{H}_{28}$ was not affected by the flow-rate of the carrier gas up to 150 ml/min and for temperatures between 343 and 393 $^{\circ}\text{K}$.

For two columns (1 and 5), the amount of stationary phase remaining at the end of the experiment was determined by combustion. The difference between the combustion results and the V_1 value at the end of the experiment calculated from eqn. 6 was +0.5% and -0.7%, respectively. This is well within the confidence interval for the determination of the loading by the combustion procedure. The factor $(dV_N/dV_{acc}) \cdot (1/K_1)$, which is a measure of the bleeding of the stationary phase, was slightly higher with benzene than with n -heptane as the solute. A possible explanation for this behavior is a small, continuously changing, side effect, caused by adsorbed water or the faster bleeding of a more polar substance such as octadecene present as an impurity in the stationary phase. This is also reflected in the different pressures obtained with the two solutes.

The means of the pressure determinations for octadecane were calculated to be 1.29 ± 0.36 and 1.46 ± 0.41 Pa with n -heptane and benzene, respectively, as solutes. (The intervals are for the 95% confidence level and the pressure values were treated as five independent observations with the same variance.) In Fig. 3 literature values for the logarithm of the vapour pressure of octadecane at 303–313 $^{\circ}\text{K}$ (ref. 15) and 393–454 $^{\circ}\text{K}$ (ref. 16) are plotted against reciprocal temperature. It can be seen that the pressures for octadecane at 333 and 371 $^{\circ}\text{K}$ determined with n -heptane and

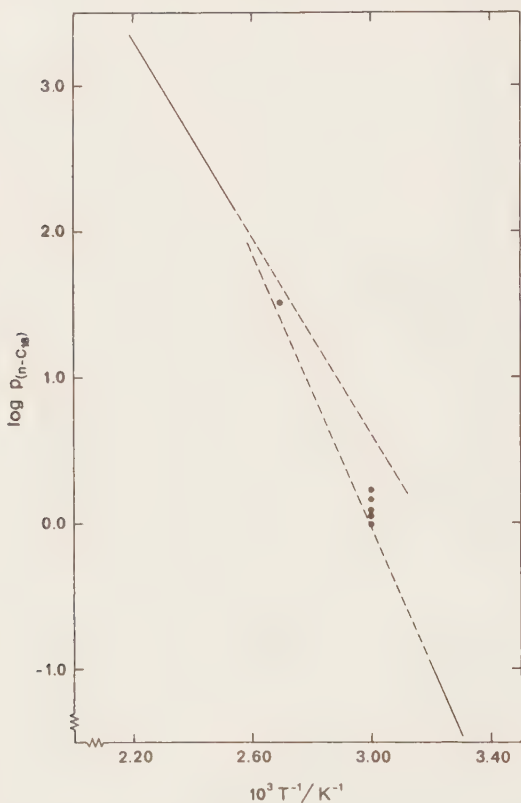


Fig. 3. Literature values for the logarithm of the vapour pressure for octadecane *versus* the reciprocal temperature^{15,16}. ●, Experimental values using *n*-heptane and *n*-nonane as solutes (this work).

n-nonane as reference solutes and calculated from eqns. 7 and 14 agree well with the available literature vapour pressures extrapolated to our temperatures. *n*-Nonane was used at 371 °K since *n*-heptane gave too low a retention volume at this temperature.

For the ODPN column, the vapour concentration has been determined to be 10^{-6} g/ml at 376 °K by Dimitrov *et al.*¹⁷ which corresponds to a vapour pressure of 25 Pa in good agreement with the value of 26.4 Pa and 373 °K determined in this investigation. The results show that the carrier gas has been saturated with the vapour of the stationary phase, and also demonstrate the very good stability of the equipment, since a small percentage decrease in V_1 over several days can be adequately measured.

CONCLUSIONS

We have shown that solute retention volume can be used for correcting the change in phase volume, due to column bleeding, and for the determination of vapour pressure of polar and non-polar phases.

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A GLC-method for the Determination of Low Vapour Pressures Applied to 1-Chloroalkanes

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A high-precision gas chromatographic apparatus coupled on line to a computer has been used for the determination of the vapour pressure of 1-chlorotetradecane, 1-chlorohexadecane, and 1-chlorooctadecane in the temperature range 40–120 °C. The chloroalkanes were used as stationary phases and the vapour pressures were calculated from the loss of the stationary phase, when a known volume of carrier gas had passed through the column. Frequent measurements were made of the retention volume for a test solute. The decrease in the volume of the stationary phase was calculated from the decrease of the retention volume for the test solute. Values of the enthalpy of vaporization were calculated from the variation of the vapour pressure with the temperature. These values were in good agreement with those determined from calorimetric measurements.

At carrier gas flow rates normally used in gas-liquid chromatography the carrier gas leaving the column is saturated with the vapour of the stationary phase.¹ The loss of stationary phase as a function of carrier gas volume can be used to calculate the vapour pressure of this phase at the column temperature. According to equations developed earlier¹ the vapour pressure p of the stationary phase is given by eqn. (1), where V_N is the net

$$p = - \frac{dV_N}{dV_{acc}} \times \frac{w_1^B}{V_N^B} \times \frac{RT}{M} \quad (1)$$

retention volume for a solute. V_{acc} is the carrier gas volume that has passed through the column from the start of the experiment. The retention volume at the start V_N^B is the intercept of the regression line for V_N versus V_{acc} . dV_N/dV_{acc} is the

slope of this line, w_1^B is the mass of the stationary phase at the start of the experiment, R is the gas constant, T the column temperature, and M the molecular weight of the stationary phase. Eqn. 1 is valid if contributions of adsorption effects to the retention volume can be neglected. This can easily be achieved by proper selection of solute. Use of silanized support and a high stationary phase loading (e.g. 20 % w/w) also minimize adsorption effects.

In this paper the vapour pressures for three chloroalkanes were determined. Chloroalkanes have previously been investigated at the Thermochemistry Laboratory in Lund, where the enthalpies of vaporization for several haloalkanes have been determined from calorimetric measurements.²

EXPERIMENTAL

All measurements were performed using a high-precision gas chromatograph coupled on line with a mini-computer. This system developed by Jönsson and co-workers^{3,4} is described elsewhere.

1-Chlorooctadecane and 1-chlorohexadecane, *puriss.* grade (Fluka AG, Buchs SG, Switzerland) and 1-chlorotetradecane, technical grade (Eastman Kodak Co, Rochester, N.Y. USA) were further purified according to the procedure described in Ref. 2, i.e. adsorption chromatography on a column of silica gel with hexane as eluent and evaporation of the solvent. Three V-shaped columns (700 × 4 mm I.D.) were used, each containing ca 3 g of packing loaded with about 20 % w/w of one of the long-chain chloroalkanes as stationary phase. Chromosorb W-AW DMS 80–100 mesh (Johns-Manville, USA) was used as the support. The carrier gas was hydrogen and methane was used for

the determination of the void volume. The carrier gas flow rate was about 50 ml/min in all experiments. This flow rate is low enough to yield complete saturation with the vapour of the stationary phase.¹

The column temperature was kept within $\pm 0.05^\circ\text{C}$ by means of an oil thermostatic bath.

It is necessary to keep the temperature of the carrier gas at column inlet and outlet the same as the column temperature. The amount of stationary phase was carefully determined by weighing at the time of the preparation of the column packing. After an experiment at one temperature, the loss of stationary phase was calculated and the remaining weight was used as the start weight (w_1^B) in the next experiment.

RESULTS AND DISCUSSION

For each temperature the net retention volume of the solute, V_N , was plotted *versus* the accumulated carrier gas volume, V_{acc} . Linear relationships were obtained. In Fig. 1 an example of such a plot is shown. Experimental conditions and results are given in Table 1 with the vapour pressures calculated according to eqn. (1).

The main cause of uncertainty in the calculation of p from eqn. (1) arises from the uncertainty in the slope dV_N/dV_{acc} of the regression line. The other contributions to uncertainty are neglected so the

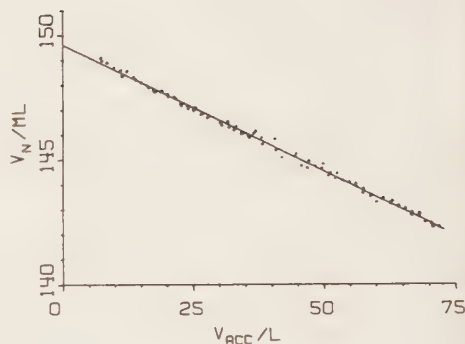


Fig. 1. Net retention volume for 1-chloropentane on a 1-chlorohexadecane column at 80.0°C *versus* the total accumulated carrier gas volume.

relative uncertainty of p and dV_N/dV_{acc} is the same. The uncertainties for p in Table 1 are given as twice the standard deviation. Boublik, Fried and Hala⁵ have calculated Antoine equations for 1-chlorotetradecane and 1-chlorohexadecane based on measurements by Kemme and Kreps⁶ in the temperature intervals $142\text{--}297$ and $165\text{--}327^\circ\text{C}$, respectively. Vapour pressures calculated with these equations are given in Table 1. They show good agreement with our values keeping in mind that extrapolations over about 100°C are involved.

Table 1. Experimental conditions and results. a = 1-chlorobutane, b = 1-chloropentane. Uncertainties are given as twice the standard deviation.

Stationary phase	$t/^\circ\text{C}$	Solute	No. of inj.	w_1^B/g	V_{acc}/l	p/Pa^a	p/Pa^b
$\text{C}_{14}\text{H}_{29}\text{Cl}$	40.1	a	100	0.4021	195	0.66(1)	0.55
	60.0	a	130	0.5024	65	4.06(6)	3.72
	80.0	b	43	0.4803	20	19.3(5)	18.6
	100.0	b	42	0.4480	8	74.0(10)	73.2
$\text{C}_{16}\text{H}_{33}\text{Cl}$	40.1	a	113	0.4190	270	0.069(7)	0.062
	60.0	b	88	0.5080	143	0.56(1)	0.55
	80.0	b	97	0.4500	73	3.61(7)	3.45
	100.0	b	61	0.5368	20	16.8(5)	16.4
	120.0	b	58	0.5007	10	63.3(8)	62.1
$\text{C}_{18}\text{H}_{37}\text{Cl}$	60.0	b	129	0.5781	463	0.111(3)	
	80.0	b	129	0.5559	290	0.78(1)	
	100.0	b	51	0.5727	44	4.10(12)	
	120.0	b	56	0.5293	33	17.0(5)	

^a This work. ^b Calculated with the Antoine equations in Ref. 5.

For each column the different temperatures were run in random sequence as can be seen from the w_1^B values in Table 1. In this way it was possible to minimize systematic errors due to long-term drift and possible error in the w_1^B -calculations. The w_1^B -calculation from one temperature to the other was checked by repeating the measurements at 60 °C on the 1-chlorohexadecane column after using the column at three other temperatures. The partition coefficient K for the distribution of the solute between the liquid and the gas phase was then calculated from $K = V_N^B \delta / w_1^B$ where δ is the density of 1-chlorohexadecane. The K -values at the two measurements were 554.1 and 553.9 and the vapour pressures 0.56 and 0.55 Pa. The value of $\delta = 0.8582 \text{ g/cm}^3$ for 1-chlorohexadecane was obtained from the value at 25 °C (Ref. 7) assuming the same volume expansion with temperatures as for 1-chlorooctadecane in Ref. 8. The validity in the procedure above of obtaining w_1^B has previously been established by combustion experiments of octadecane columns.¹ The influence of impurities on the vapour pressures of the chloroalkanes is different for impurities with considerable higher or lower vapour pressure than the investigated chloroalkane. Those with lower vapour pressure will give only small errors in the vapour pressure since the vapour pressure will be proportional to the mol fraction of the main component according to Raoult's law. Thus the relative error will be approximately the same as the impurity concentration. Impurities with higher vapour pressure will affect the slope of the line of V_N versus V_{acc} only in the beginning of the first experiment on a column. Consequently the experiment should be run long enough for a straight line to be obtained, which may not be the case for a completely new column. However, in the systems described in this work, such effects were not observed.

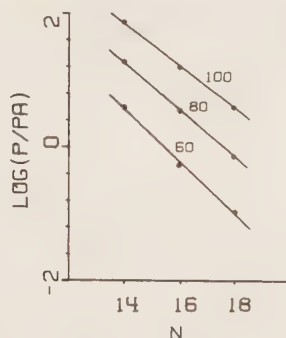


Fig. 2. The logarithm of the vapour pressure for 1-chloroalkanes with N carbon atoms.

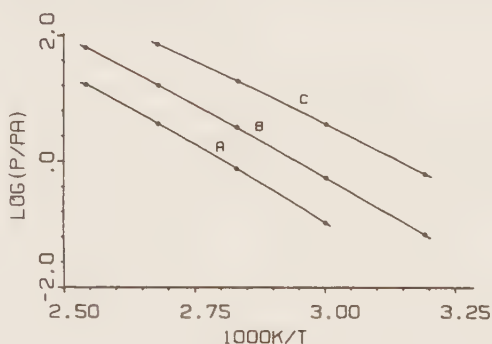


Fig. 3. The logarithm of the vapour pressure for 1-chloroalkanes versus the reciprocal temperature. A = 1-chlorooctadecane; B = 1-chlorohexadecane; C = 1-chlorotetradecane.

Plots of $\log p$ at certain temperatures versus the number of carbon atoms, N , in the chloroalkanes are given in Fig. 2. As expected, these plots are linear. Plots of $\log p$ versus the reciprocal temperature appear as slightly curved lines (Fig. 3).

Table 2. Enthalpies of vaporization, ΔH_{vap} (in kJ mol^{-1}), for 1-chloroalkanes.

$t/^\circ\text{C}$	1-chlorotetradecane	1-chlorohexadecane	1-chlorooctadecane
25	81.8 ^a	91.5 ^a	101.2 ^a
40	80.2	91.2	—
60	78.0	92.7	96.9
80	74.4	87.0	93.4
100	72.9	81.5	88.4
120	—	82.1	86.7

^a From Ref. 2.

The enthalpy of vaporization is calculated according to the Clausius-Clapeyron equation⁹ which can be written:

$$\frac{\Delta H_{\text{vap}}}{R} = - \frac{d \ln p}{d(1/T)} \quad (2)$$

To calculate ΔH_{vap} it is necessary to numerically differentiate the relations represented in Fig. 3. To accomplish this, spline functions (with a knot at every data point)¹⁰ were fitted to the experimental data. The slightly curved lines in Fig. 3 are obtained from these functions. The vaporization enthalpies were then calculated from these functions according to eqn. (2) and are given in Table 2 and Fig. 4.

ΔH_{vap} -values for some chloroalkanes at 25 °C were determined at the Thermochemistry Laboratory.² An empirical equation of ΔH_{vap} at 25 °C as a function of the number of carbon atoms in the chloroalkanes was given. Values calculated by this equation are shown in Table 2 and Fig. 4. Regression lines for ΔH_{vap} versus temperature were calculated from the ΔH_{vap} -values determined by us for 1-chlorotetradecane and 1-chlorooctadecane. For 1-chlorohexadecane no regression line was calculated because of the large scatter of the points (see Fig. 4). The regression lines gave values of ΔH_{vap} at 25 °C in good agreement with those in Ref. 2. Including these literature values new regression lines were calculated. The change of heat capacity ΔC_p is the slope of the respective line. Table 3 contains ΔC_p values for 1-chlorotetradecane and 1-chlorooctadecane together with the

Table 3. ΔC_p values for the vaporization of chloroalkanes and normal alkanes.

Substance	$\Delta C_p/\text{kJ mol}^{-1} \text{K}^{-1}$
1-Chlorotetradecane	0.12
1-Chlorooctadecane	0.16
n-Tetradecane	0.11 ^a
n-Octadecane	0.15 ^a

^a Extrapolated from Ref. 11.

values for the corresponding n-alkanes as a comparison. These were obtained by linear extrapolation from literature data for lower homologs.¹¹

The method developed above is best suited for the determination of vapour pressures in the range 0.1–100 Pa. For higher vapour pressures the fast bleeding of the stationary phase gives rise to disturbances in the detector function, especially when using a flame ionization detector. At very low vapour pressures the long-time stability of the equipment will be a problem. The use of an on-line computer has of course facilitated the measurements but we think that similar experiments can be performed on a commercial chromatograph equipped with a good timer provided the carrier gas flow is well-known at each injection of solute.

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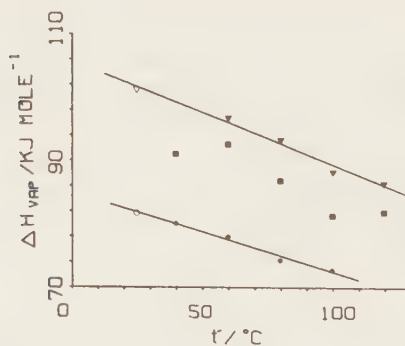


Fig. 4. Heat of vaporization for 1-chloroalkanes versus temperature.

○, ● = 1-chlorotetradecane; □, ■ = 1-chlorohexadecane; △, ▲ = 1-chlorooctadecane; Filled symbols: This work; Unfilled symbols: Ref. 2.

VI

DETERMINATION OF THE VAPOUR PRESSURES OF SOME MOTH SEX
PHEROMONE COMPONENTS AND RELATED COMPOUNDS BY A GAS
CHROMATOGRAPHIC METHOD

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Introduction

The volatility, measured as the saturated vapour pressure, of sex attractant pheromone components is an important factor in studies on the physico-chemical aspects of insect sex pheromones. Hirooka and Suwanai (1976) have, for instance, derived an equation which relates the rate of pheromone release by a female moth to the vapour pressure, the diffusion coefficient and the size and form of the pheromone gland. For lepidopterous species the pheromone generally is a mixture of several compounds, mainly olefinic acetates with varying chain lengths. In many cases also geometrical and/or positional isomers are present in the pheromone blend (Ritter, 1979). The female moths produce a well defined ratio of the different compounds and the component ratio

has also been found to play an important role in attempts to trap males in the field (Roelofs, 1978). However, when the vapour pressures of the pheromone components differ significantly, the ratio of the components on the female gland must be different from that in the gas phase. The latter ratio depends on the relative saturated vapour pressures of the components and their relative mole fractions in the liquid phase (Hirooka and Suwanai, 1978).

Knowledge of the vapour pressures of pheromone components and related compounds may also be important in electrophysiological studies. In a typical experiment a certain amount of the test compound is loaded on to a piece of filter paper which is placed in an odour cartridge (O'Connell, 1975). The saturated vapour pressure is allowed to build up and part of the vapour phase is then carried by an air current onto the antennal preparation. Normally reference is made to the amount of chemical loaded onto the filter paper. For accurate studies of, for instance, dose-response relationships, the actual amount of the test compound that is used in the experiment may easily be calculated, provided that the saturated vapour pressure of the compound is known. This may be of importance if the electrophysiological activity of molecules with significantly different vapour pressures are to be compared.

Although the vapour pressures of the compounds used by insects as sex pheromone components enter into many different types of pheromone studies, very few experimental values are available. Hirooka and Suwanai (1978) used a gas satura-

tion method to determine the vapour pressure of a few compounds used as pheromone components by lepidopterous species.

In this paper we wish to report on vapour pressures for some pheromone components and related compounds determined by a gas chromatographic method, developed by two of us (Olsson *et al*, 1976). It has been used for several applications (Jönsson *et al*, 1980, Jönsson and Pscheidl, 1981). This method is suitable for this work as it is possible to make measurements at biologically relevant temperatures. The demand on sample substance is small and the main part can be recovered.

Four of the compounds chosen for this work, decyl, (Z)-5-decenyl, (Z)-7-dodecenyl and (Z)-9-tetradecenyl acetate, have been identified as sex pheromone components of the turnip moth, *Agrotis segetum*, (Tóth *et al*, 1980, Arn *et al*, 1980 and Löfstedt *et al*, 1981). To investigate the influence of the position of the double bond on the vapour pressure, (Z)-3-, (Z)-4-, and (Z)-6-decenyl acetate were included in the study. Finally the vapour pressure of an geometrical isomer, (E)-5-decenyl acetate, was determined.

Methods and materials

Principles The substance under study is used as the stationary liquid phase (SLP) in a gas-liquid chromatographic column. A suitable sample compound, the "probe", is repeatedly injected into the gas chromatograph and the net retention volume V_N is carefully measured. According to basic gas

chromatographic theory, the following equation applies:

$$V_N = V_R - V_M = K \cdot V_L \quad (1)$$

Here V_R is the total retention volume, usually calculated as the product of the retention time and the carrier gas flow rate. V_M is the volume of the empty space in the column, V_L is the volume of the SLP and K is a constant, the partition coefficient. V_M can be measured as the retention volume of methane as K for methane can be neglected under normal conditions.

Due to the evaporation of the SLP, V_L decreases linearly with the total amount of gas, V_{acc} , which has passed the column since the start of the experiment. As is seen from eq.(1), this means that V_N also decreases in the same way. An experimental example of such a line is shown in fig. 1.

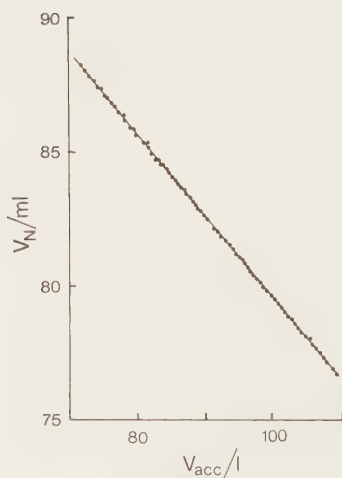


Fig. 1. The retention volume V_N of *i*-buthyl acetate on a (Z)-7-dodecenyl acetate column vs the accumulated carrier gas volume V_{acc} at 35.1° C.

The slope, $-dv_N/dv_{acc}$ is proportional to the evaporation rate and thus to the vapour pressure of the SLP if the carrier gas is saturated with the evaporated substance. This is normally the case which was shown by Olsson and coworkers (1976) and Olsson and Jönsson (1981).

From a knowledge of the initial weight of the SLP w_L^B and the initial retention volume V_N^B , the vapour pressure p of the SLP can be calculated from the equation (Olsson *et al*, 1976):

$$p = - \frac{dv_N}{dv_{acc}} \cdot \frac{w_L^B}{V_N^B} \cdot \frac{R \cdot T}{M} \quad (2)$$

Here, T is the absolute temperature, M is the molecular weight of the SLP and R is the gas constant. Eqs. (1) and (2) are valid if contributions from adsorption effects to the retention volume of the probe can be neglected. This can be achieved by selection of a probe which is chemically similar to the SLP.

Chemicals The following acetates were used in the study: decyl (10:Ac), (Z)-3-decenyl (Z3-10:Ac), (Z)-4-decenyl (Z4-10:Ac), (Z)-5-decenyl (Z5-10:Ac), (E)-5-decenyl (E5-10:Ac), (Z)-6-decenyl (Z6-10:Ac), (Z)-7-dodecenyl (Z7-12:Ac) and (Z)-9-tetradecenyl (Z9-14:Ac).

The monoolefinic compounds were all synthesized from alkynes and ω -bromoalkyl tetrahydropyranyl ethers. The resulting alkynyl tetrahydropyranyl ethers were hydrogenated over

Lindlar catalyst to give the corresponding (Z)-alkenyl compounds or were treated with sodium in liquid ammonia to give the (E)-alkenyl compound. Finally they were converted to acetates by acetyl chloride in acetic acid.

After purification by argentation chromatography using a cation-exchange resin impregnated with silver nitrate (Houx *et al*, 1974) the compounds were >99.5 % pure except for Z9-14:Ac which was about 97 % pure.

Experimental procedure For each pheromone substance, one gas chromatographic column was prepared, according to the method described by Conder and Young (1979, p.595). The solid support was Chromosorb W-AW-DMCS (Johns-Manville, Denver, Colorado). About 600 mg of packing, containing about 60 mg of pheromone liquid was filled into each column. The columns were made by glass, V-shaped, and had the dimensions 800 mm x 2 mm I.D. The initial amount of liquid was determined by careful weighings during the column preparation procedure.

All retention volume measurements were performed using a high precision gas chromatograph, coupled on-line with a mini-computer. This system which was described in detail elsewhere (Jönsson *et al*, 1975) permits the direct measurement of retention volumes, without involving the concept of retention time.

The carrier gas was hydrogen, the flow rate of which was ca 30 ml/min. The column volume V_M , was measured by injection

of methane (99.95 %). As "probe" we used *i*-butyl acetate (Eastman, Rochester, N.Y.) which is chemically similar to the pheromone substances studied, thus minimizing adsorption. This is shown by the symmetric shape of the chromatographic peaks obtained.

Vapour samples of the probe were repeatedly injected at specified time intervals under computer control. For each injection the retention volume was automatically calculated by the computer and stored on flexible discs for further computer processing. The evaporation of a substance was traced at each temperature, until the 95 % confidence interval for the slope dV_N/dV_{acc} was less than 1 % relative. This is of the same magnitude as other sources of error, and, consequently, the precision cannot be improved by further measurements. Depending on the vapour pressure, the time needed to reach enough high precision was 5 to 75 hours. The same column was used for several temperatures as in an earlier work (Jönsson *et al*, 1980) and the temperatures were run in random order.

Results and discussion

In table I and figures 2 and 3 the results of the vapour pressure measurements are summarized. In the figures, the value of $\ln p$ are plotted *vs* the reciprocal temperature. In a limited temperature interval, a straight line for each substance should be obtained.

Table I

Vapour pressures p for the pheromone substances studied. The uncertainty is given as a 95 % confidence interval. The abbreviations of the names of the substances are explained in the text under "chemicals".

Substance	Temp ($^{\circ}\text{C}$)	p (Pa) [¶]
10:Ac	25.9	2.48 ± 0.01
	26.1	$2.52 \pm 0.04^*$
	30.4	3.80 ± 0.05
	34.8	5.66 ± 0.03
	39.4	8.86 ± 0.03
	39.5	$8.70 \pm 0.11^*$
Z3-10:Ac	26.0	3.02 ± 0.04
	30.4	4.65 ± 0.04
	34.8	6.76 ± 0.07
	39.4	10.40 ± 0.11
Z4-10:Ac	34.9	8.26 ± 0.06
	34.9	$7.95 \pm 0.06^*$
	39.4	12.12 ± 0.09
Z5-10:Ac	26.2	2.93 ± 0.04
	30.6	4.65 ± 0.04
	35.1	6.91 ± 0.06
	39.6	10.13 ± 0.11
E5-10:Ac	26.0	2.84 ± 0.02
	30.4	4.08 ± 0.04
	34.9	6.44 ± 0.08
	39.3	9.58 ± 0.10
Z6-10:Ac	25.9	2.91 ± 0.03
	30.4	4.38 ± 0.03
	34.8	6.83 ± 0.06
	39.4	10.08 ± 0.06
Z7-12:Ac	30.5	0.562 ± 0.005
	34.8	0.83 ± 0.01
	35.1	$0.89 \pm 0.01^*$
	39.2	1.33 ± 0.01
	44.0	2.01 ± 0.02
	44.1	$2.07 \pm 0.01^*$
Z9-14:Ac	30.5	0.094 ± 0.001
	35.0	0.163 ± 0.001
	39.5	0.267 ± 0.002
	44.0	0.428 ± 0.004

* New, independent measurement. See text.

[¶] $1 \text{ Pa} = 0.987 \cdot 10^{-5} \text{ atm} = 7.50 \cdot 10^{-3} \text{ mm Hg}$.

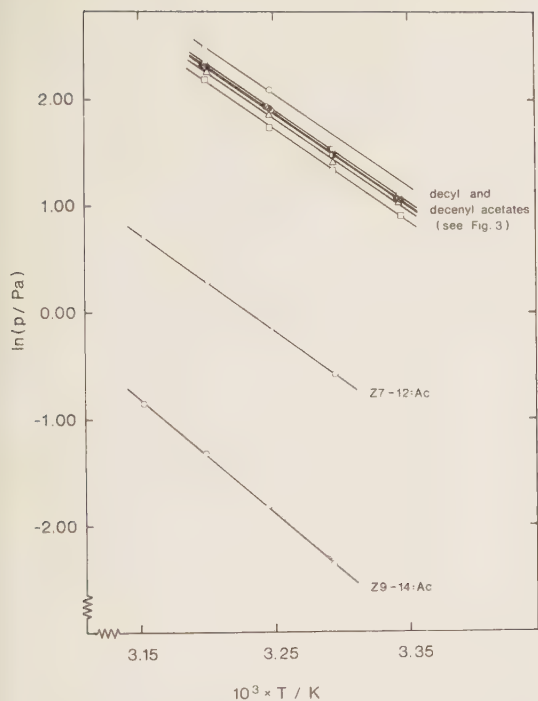


Fig. 2. The logarithm of the vapour pressure for pheromone substances vs the reciprocal temperature. The abbreviations for the names of the substances are explained in the text under "Chemicals".

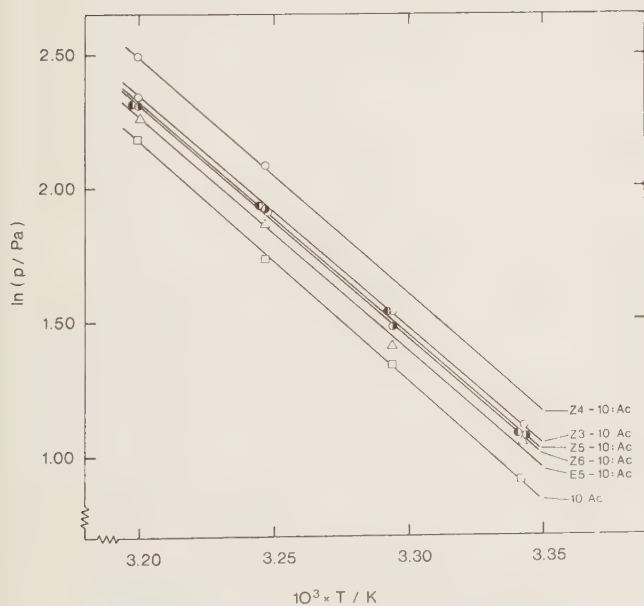


Fig. 3. Expansion of the upper part of fig. 2.

In a few cases the measurements were repeated with a new column. Such data are marked with an asterisk in the table and are not plotted. It can be seen that repeated measurements give values which closely agree with each other. This reflects the good reproducibility in the entire measurement procedure, including the preparation of the column.

In an earlier study (Jönsson *et al*, 1980) vapour pressures for 1-chloroalkanes were found to agree well with literature data, generally within 5 %, and we conclude that the method gives reliable vapour pressure values.

In the figures 2 and 3 we see that the slopes of the lines for all decyl and decenyl acetates are approximately the same and that the slope increases with increasing chain length. From this slope, the enthalpy of vaporization ΔH_{vap} , can be calculated according to the Clausius - Clapeyron's equation:

$$\frac{d \ln p}{d(1/T)} = - \frac{\Delta H_{\text{vap}}}{R} \quad (3)$$

The results calculated from our data are: 72 kJ mole⁻¹ for decyl and decenyl acetates, 77.5 kJ mole⁻¹ for Z7-12:Ac and 90 kJ mole⁻¹ for Z9-14:Ac. These data agree well with vaporization enthalpy data obtained for 1-chloroalkanes of similar molecular sizes (Jönsson *et al*, 1980).

Hirooka and Suwanai (1978) measured vapour pressures for similar compounds as in this study. Only one of them is the same (Z9-14:Ac). The agreement with our values for this

compound is within 10 % at 40°C and 50°C, but at 30°C their value is ca 50 % higher than ours. This compound is one of the least volatile in their study. The vaporization enthalpy for Z9-14:Ac calculated from the data of Hirooka and Suwanai is 72 kJ mole⁻¹, which seems to be unrealistically low. Their method seems to suffer from systematic errors, especially at low vapour pressures.

No other sources of literature data of vapour pressures at biologically relevant temperatures for the substances under study have been found, not even in a very comprehensive and modern compilation (Dykyj and Repáš, 1979).

Effects of the position of the double bond From measurements on computer generated models of the different (Z)-decenyl acetates, the mid-point of the molecule is found to be near the 4-position. Thus Z4-10:Ac is the most symmetrical compound with respect to the double bond position. As (Z)-olefines are folded at the double bond, a more centrally located double bond gives a smaller molecular size. The vapour pressure mainly depends on intramolecular forces in the liquid phase. Therefore, a molecule with a smaller effective size, should have a higher vapour pressure than one which is effectively larger. Thus Z4-10:Ac will have the highest vapour pressure, followed by Z3-10:Ac and Z5-10:Ac which is in agreement with the data obtained.

This effect can also be seen in literature data for small olefines as hexenes (Camin and Rossini, 1956) and heptenes (Eisen and Orav, 1970). (Z)-3-Hexene and (Z)-3-heptene both

have higher vapour pressures than (Z)-2-hexene and (Z)-2-heptene, respectively.

Also, Butler and Mc Donough (1979) found higher evaporation rates for more symmetric Z-olefine acetates from rubber septa. This was explained by less retardation by the rubber matrix. Our results indicate that at least part of this effect may be explained by differences in vapour pressure.

Effects of the chain length The vapour pressure rates at 35°C for the homologs studied in this work are 7.8 for Z5-10:Ac/Z7-12:Ac and 5.4 for Z7-12:Ac/Z9-14:Ac. Thus there is no strict additivity in $\ln p$ for the homologs in this series as is found for more simple compounds, e.g. normal alkanes. As was shown above, the vapour pressures of olefinic compounds depend on the position of the double bond. Strict additivity should thus only be expected for a series of homologs in which the compounds have closely the same relative length of the two hydrocarbon chains separated by the double bond.

Effects of the double bond configuration In the only pair which we have studied, Z5-10:Ac has a higher vapour pressure than E5-10:Ac. The difference is less than 10 %. From the literature data on hexenes and heptenes (see above) it can be seen that the relation between the vapour pressures for E-forms and for Z-forms vary with the position of the double bond.

Effects of a double bond All decenyl acetates have higher

vapour pressures than the saturated decyl acetate. This is a normal and expected case.

Acknowledgement

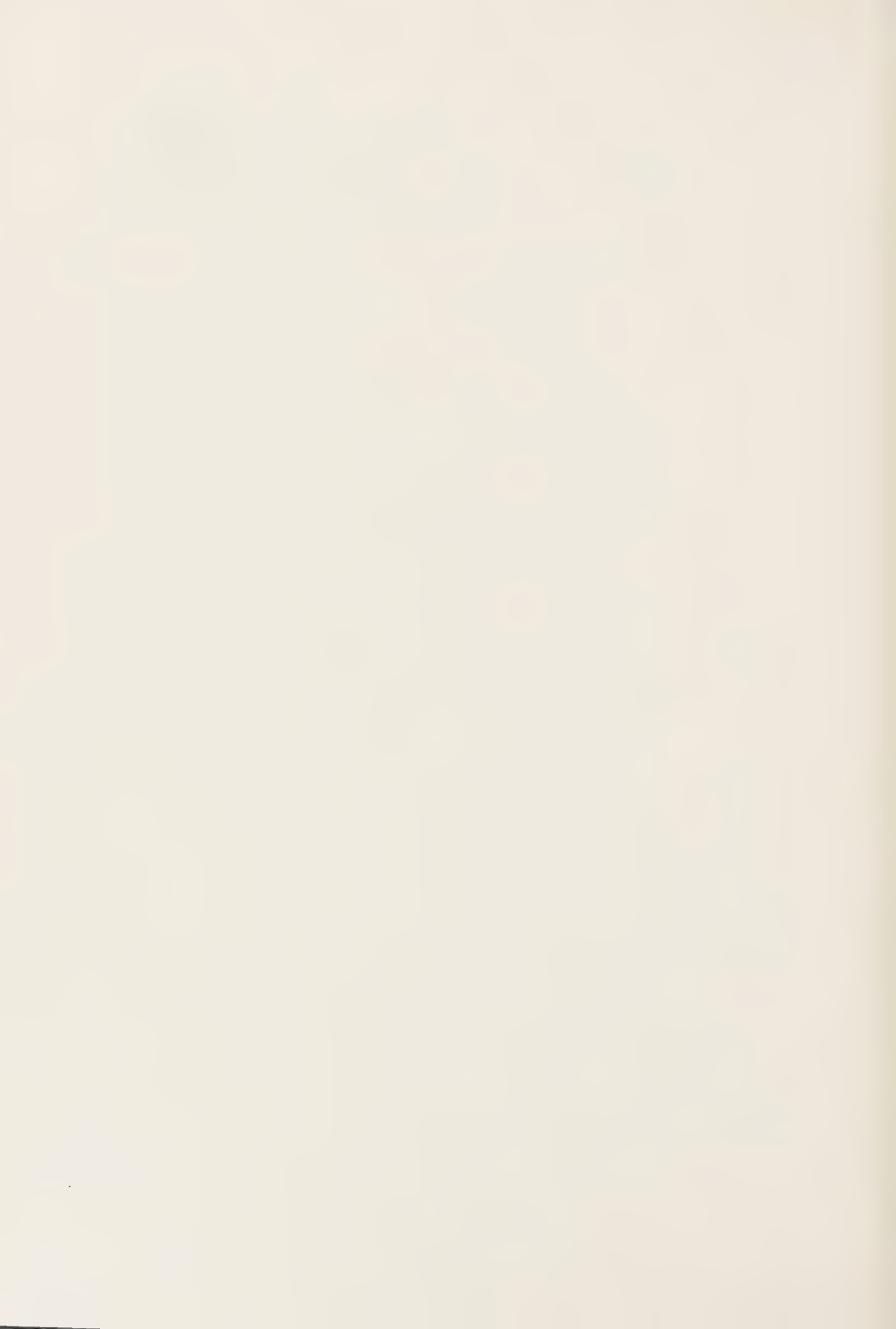
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VII



DISTRIBUTION OF THE STATIONARY PHASE IN THE GLC-COLUMN

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INTRODUCTION

The determination of the liquid phase volume creates the greatest source of error in GLC-measurements of thermodynamic and related quantities [1]. A problem of a similar nature, however less studied, is the distribution of the liquid phase in the column. Theories for the relation between the theoretical plate height and liquid film thickness assume that the liquid is evenly spread on the support surface, which in reality is not true. Theoretical considerations of the liquid phase arrangement on the solid support structure was made by Giddings [2], who derived relationships for the influence of an uneven liquid film thickness on the theoretical plate height. From these relationships it can be seen that the highest efficiency is obtained when the liquid is homogenously spread on the surface. Furthermore, the measurements of diffusion coefficients [3], demand values for the film thickness and uneven liquid distribution makes such measurements difficult to interpret.

The arrangement of the liquid phase created at the time of

preparation of the packing is changed by various processes. During the conditioning of the column, the liquid is redistributed within a support particle, by the action of adsorption and capillary forces, towards an equilibrium configuration. Additionally, secondary redistributions of the liquid, on a column scale, can be considerable and are relatively little described. The evaporation of relatively volatile stationary phases diminishes the liquid content in the beginning of the column, as was shown by Keller and co-workers [4]. This process can produce uncovered support patches, increasing adsorption of the analyte and impairing the resolution. It changes the film thickness and thus the efficiency. Also, in kinetic studies involving a non-volatile catalyst in a relatively volatile solvent as the stationary liquid phase, this effect can cause considerable problems [5].

There is also the possibility that the liquid phase flows in the column due to gravity, thus producing a thicker liquid film in the parts of the column which are lowest situated.

To study some of these effects, two columns, primarily used for the determination of the vapour pressure of different substances [6,7] were cut into pieces and the liquid content in each part was measured.

EXPERIMENTAL

Two V-shaped columns made of glass, designated A and B, were

studied. They were both used in the precision gas chromatograph described earlier [8].

Column A:

This column [6] had the dimensions 1000 mm x 4 mm I.D. It was filled with a packing consisting of Supasorb (AW-HMDS) 40-60 mesh (BDH, Poole, G.B.) coated with ca 20 % n-octadecane. The column was kept at 60°C during 51.5 hours and a total of 160 l of N₂ was passed through. Afterwards, the column was cut into seven pieces and the liquid phase content of each part was calculated from careful weighings before and after heating the packing to ca 800°C.

Column B:

This column [7] had the dimensions 800 mm x 2 mm I.D. It was filled with a packing consisting of Chromosorb W AW-DMCS (John Manville, Denver, Colorado) coated with ca 10 % of (E)-5-decenylacetate.

The bleeding of this column was studied at four successive temperatures and a total of ca 50 l of N₂ was passed through. Afterwards the column was cut into five pieces and the liquid phase content was calculated from careful weighings of the packing before and after extraction with n-hexane in a Soxhlet extractor.

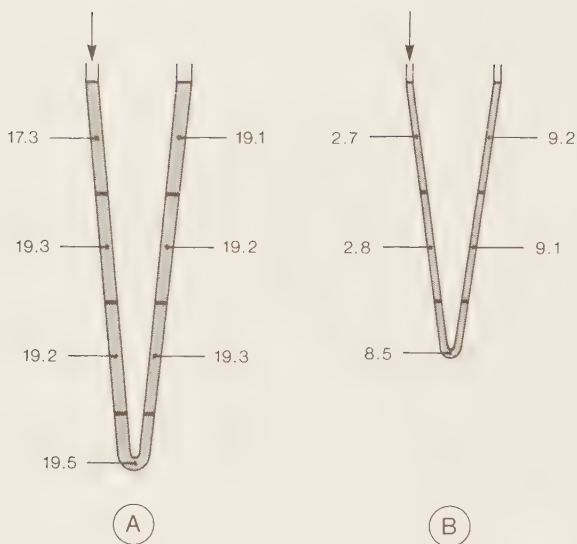


Figure 1. Liquid loading in weight percent in the columns A and B after use as described in the text. Initial loadings: for A, 19.42 %, for B 10.03 %.

RESULTS AND DISCUSSION

The results of the experiments are shown in fig. 1. We can see that the depleting of the stationary phase from the inlet end occurred as Keller and coworkers found [4]. We can also see (most clearly for col. A) that the liquid phase indeed has flown downwards, making the concentration in the middle (lowest) part of the column slightly larger.

In these experiments, the injector and detector temperatures have been kept the same as that of the column which is not the common case in practical GC. In most commercial GC

instruments, the thermal insulation is insufficient to prevent that heat flows from the detector and injector ovens and to some extent heats the ends of the column. This accelerates the loss of liquid at the inlet end, and additionally causes losses at the outlet end. The resulting uneven liquid distribution can create loss of efficiency and uncovered patches, causing adsorption of the analyte.

From the liquid distribution profile it can be seen that the gas is saturated with the vapour of the stationary liquid phase already after its passage through a short piece of the column. This is in agreement with the observation [6] that the partial pressure which can be calculated from the decrease in retention volume due to the evaporation of the stationary phase, is equal to the saturated vapour pressure.

To verify the analysis of the liquid phase content in the column parts, the following comparisons can be made:

Column A:

From the known initial amount of n-octadecane, from its vapour pressure and from the volume of the gas which has passed through the column, the expected liquid contents of the column after the experiment was calculated to 718 mg. This is in good agreement with the value found by adding the amounts found in the seven parts (719 mg).

Column B:

From the decreases in retention volume at four successive temperatures and from the known initial amount of the liquid

phase, the expected contents of the column after the experiments was calculated in the way which was described in ref. 9. The result was 34.7 mg. The sum of the contents of the five parts of the column was 35.3 mg.

Thus it can be summarized that the effects shown in fig. 1 are hardly due to imprecision in the analysis and that the conclusions are justified.

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